Supplementary Information

Microencapsulation of bacterial strains with graphene oxide nano-sheets using vortex fluidics

M. Haniff Wahid[a,b], Ela Eroglu[c], Sian M. LaVars[a], Kelly Newton[a], Christopher T. Gibson[a], Uwe H. Stroeher[d], Xianjue Chen[a], Ramiz A. Boulos[a], and Colin L. Raston[a], Sarah-L. Harmer[a]*

[a] Flinders Centre for NanoScale Science and Technology, School of Chemical and Physical Sciences, Flinders University, Bedford Park, SA 5042
[b] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[c] ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.
[d] School of Biological Sciences, Flinders University, Bedford Park, SA 5042, Australia

S1. Materials and methods

S1.2 Preparation of bacterial culture

Staphylococcus aureus (ATCC 6538) and *Rhodococcus opacus* (DSN 43205) were maintained on nutrient agar and were grown to stationary phase in Luria-Bertani and GYM *Streptomyces* medium respectively.[1,2] Before being used for VFD experiments, both strains were grown in broth culture until stationary phase, as determined by spectroscopic optical density measurements at 600 nm (Varian Cary 50 Bio UV/Visible spectrophotometer).

S1.1 Synthesis of graphene oxide

Graphene oxide used in this study was synthesized from natural graphite powder (99.9%, SP-1, Bay Carbon) using the modified Hummers method.[3,4]

S2. Study effect of VFD processing towards viability of the bacteria using flow cytometry

For flow cytometric analysis, a tube of each of the bacterial species without VFD processing was prepared as a positive control. For VFD processed bacteria, samples were prepared in triplicates. In order to establish the regions in the flow cytometric analysis that corresponded to live and dead cells, cells were heat injured at 60 °C for 1 h and used as a dead control,

whereas untreated cells were used as a live control. Cells were stained (15 min at room temperature, in the dark) with the cell-permeant double-stranded DNA fluorochrome, SYBR green I (Sigma-Aldrich) at a final concentration of the commercial stock solution of 1:10,000 (v/v) in Tris-Acetate EDTA buffer and with propidium iodide (PI) (Sigma-Aldrich) at a final concentration of 10 $\mu g.mL^{-1}$ in water.[5] SYBR stains the nucleic acids in all cells, while PI stains the nucleic acids in cells with damaged membranes. Sample analysis was performed using a LSR Fortessa flow cytometer (Becton Dickinson Biosciences, San Diego, CA, USA) equipped with an air-cooled 488-nm argon ion laser. Each cell was characterized by four optical parameters: side-angle scatter (SSC), forward-angle scatter (FSC), green fluorescence for SYBR (525 nm) and red fluorescence for PI (675 nm). Data were collected using FACS Diva Software version 8.0 supplied by BD Biosciences.

S3. Wrapping of bacteria with graphene oxide using VFD

Samples of *S. aureus* and *R. opacus* were diluted at a ratio of 1:2 (v/v) with graphene oxide suspension (0.1 $mg.mL^{-1}$ prepared in sterile MilliQ™ water), and vortexed using the VFD under aseptic conditions for one minute in triplicate. The 10 mm OD diameter NMR tube in the VFD was cleaned with 70 % ethanol and rinsed with sterile MilliQ™ water between samples. Vortexed samples were then aseptically placed in fresh media (10 % initial concentration) and incubated at 37 °C and 28 °C with a shaking speed at 155 rpm using CERTOMAT®R and Ratek shakers respectively. To determine the growth curves, optical density measurements of the aliquots were immediately taken after VFD treatment in parallel with the control at proper dilutions at every 4 hours for *S. aureus* and every 8 hours for *R. opacus* until they reach their stationary phase. Sterile media with graphene oxide particles (5 % final concentration) was used to blank the spectrophotometer.

As a control, samples of *S. aureus* and *R. opacus* (5 % of initial concentration) were cultured without graphene oxide particles and without vortexing, then incubated alongside the treated samples. Sterile media was used as a blank for the optical density measurements of the bacteria-only samples.

As a further control to determine the effect of graphene oxide without VFD processing, samples of *S. aureus* and *R. opacus* were diluted at a ratio of 1:2 (v/v) with graphene oxide (10 % of final concentration), placed in fresh media and incubated alongside the treated samples. Sterile media with graphene oxide particles (5 % of final concentration) was used as a blank for the optical density measurements of the GO treated samples.

S4. Flow cytometry analysis

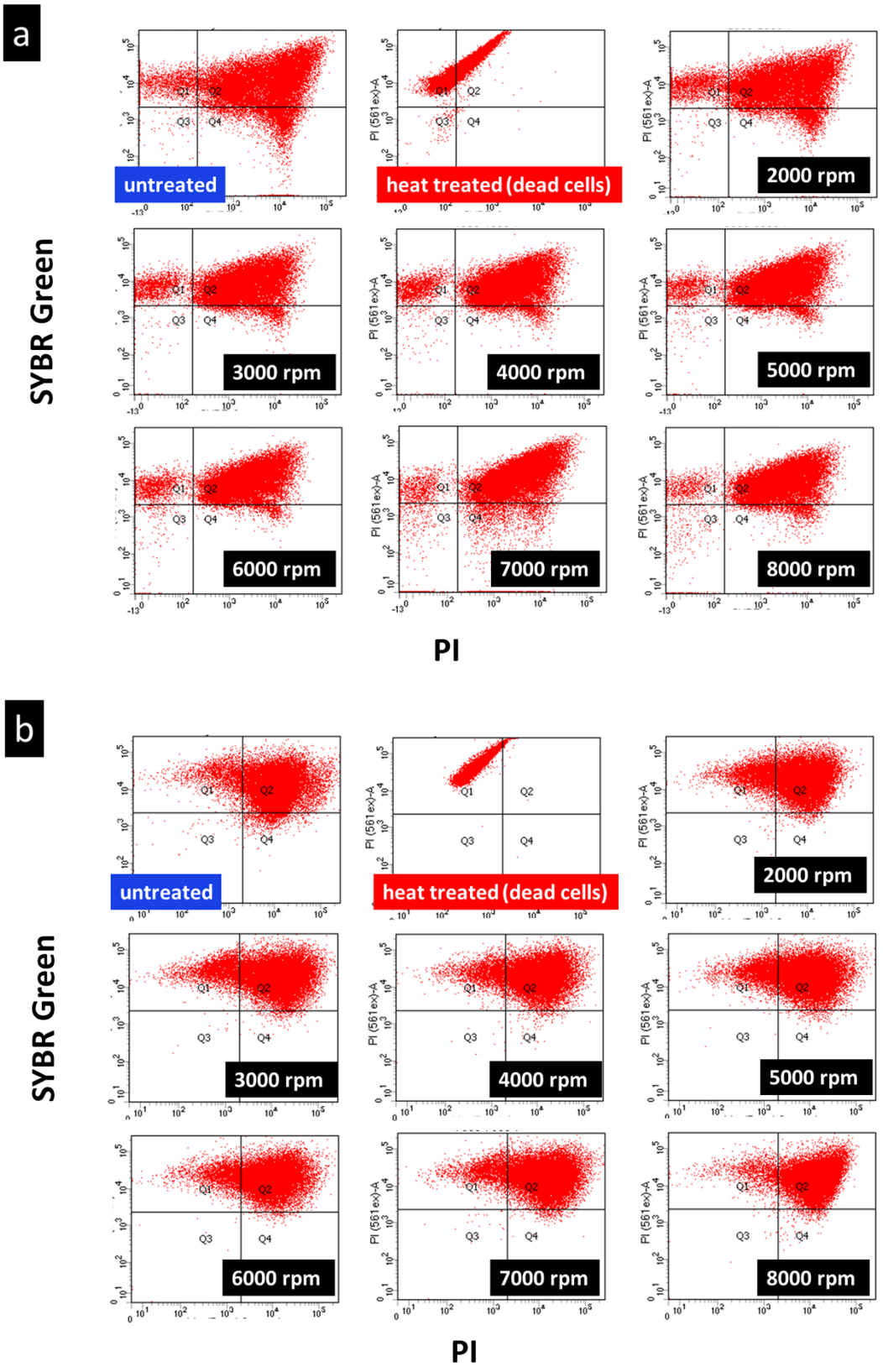

Figure S4. Flow cytometric analyses of (a) *S. aureus* and (b) *R. opacus* after processing in VFD at different speeds ranging from 2000-8000 rpm for 1 minute.

S5. AFM analysis

All AFM measurements were acquired using a Bruker Multimode AFM with a Nanoscope V controller. AFM images were acquired in tapping mode with imaging parameters, such as the set-point, scan rate and feedback gains, adjusted to optimize image quality. Images were analysed using the Nanoscope analysis program version 1.40. The AFM probes used were Mikromasch HQ:NSC15 Si probes with a nominal spring constant of 40 N/m and a nominal tip diameter of 16 nm. The scanner was calibrated in the x, y and z axes using Si calibration grids (Bruker model numbers PG: 1 μm pitch, 110 nm depth, and VGRP: 10 μm pitch, 180 nm depth).

S6. Raman analysis

Raman spectra and images were collected with a WiTEC alpha300R Microscope in confocal imaging Raman mode using 100 × (Numerical Aperture 0.9) objective with a 532 nm Nd-YAG green (E = 2.33 eV) laser operating at constant power for each experiment. Laser power was kept below 5 mW during all measurements. Spectral images were acquired using integration times between 2.5 to 5 s per pixel with images composed of between 30 x 30 and 150 x 150 pixels depending on scan size. Each pixel corresponds to a separate Raman spectrum, allowing hundreds to thousands of spectra acquired during an image scan. Raman images were generated by selecting a region in each spectrum, in which a material specific peak is observed. For graphene oxide (GO) the peak chosen was the strong graphitic band at 1600 cm^{-1}. The intensity of this selected region is plotted relative to the x, y position of the scanning laser. Single spectra were also acquired at points on the Raman images with typical integrations times between 30 s to 60 s and 2 to 3 accumulations per spectra. Raman data was collected by the WiTEC Control software and analysed using the WiTEC Project software.

S7. Bacterial cell growth monitoring using optical density measurements at 600 nm

The growth of GO wrapped bacteria was monitored using optical density (O.D) method at 600 nm (Figure S7). O.D. measurements taken from GO wrapped bacteria shows that both bacteria are still viable even after interfacing with graphene oxide. The detailed kinetic analysis of these data are given in the main text (Fig. 7, Fig. 8, Table 1).

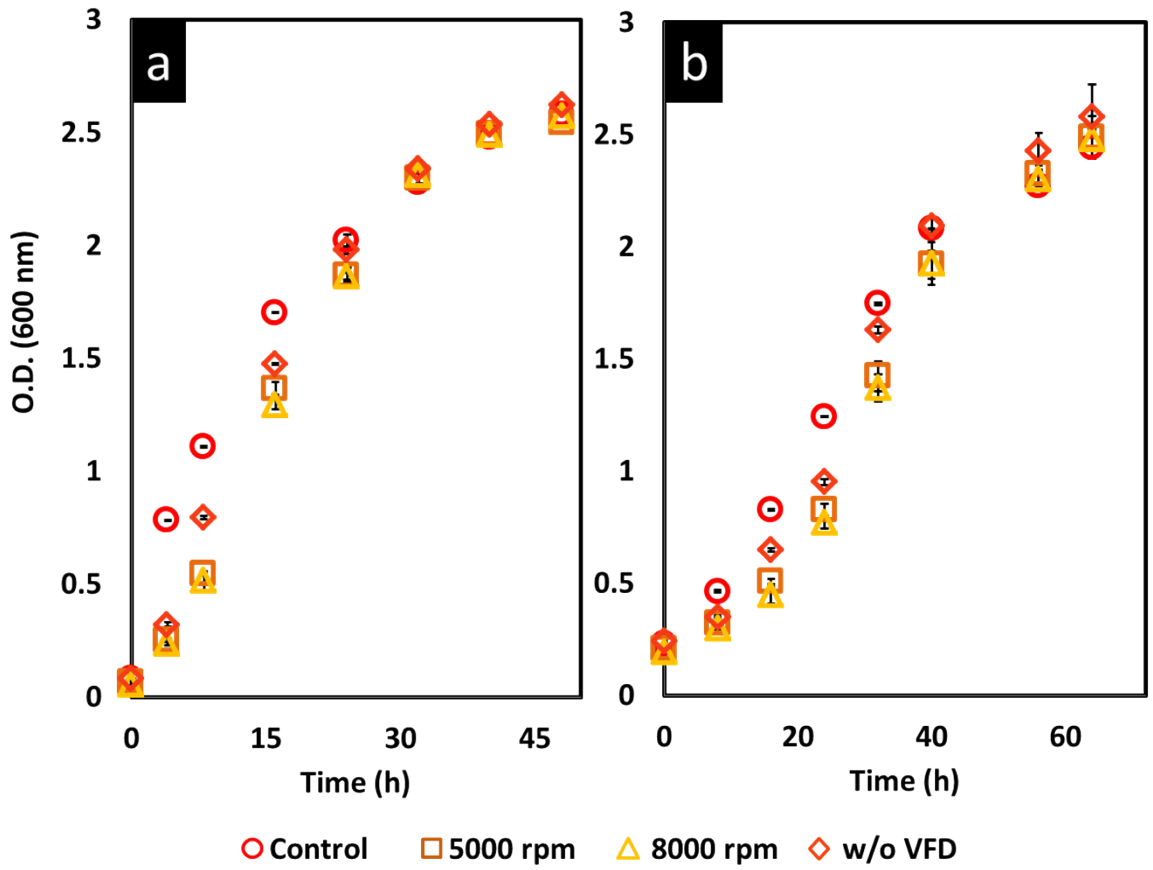

Figure S7. Optical density measurements of (a) *S.aureus*, and (b) *R.opacus* cells regrown in nutrient media after wrapping with GO (Red circle: control; brown rectangle: 5000 rpm/1 min; yellow triangle: 8000 rpm/1 min; orange diamond: w/o VFD).

S8. Calibration curve for optical density measurements at 600 nm versus viable cell count

The bacterial strains *S. aureus* and *R. opacus* were prepared by growing to an $O.D._{600}$ of ~1 in Luria-Bertani (Miller)[3] or GYM Streptomyces[4] medium, respectively. Several dilutions of the $O.D._{600}$ ~1 were prepared covering a wide region of optical density from 0.02 to 1. $O.D._{600}$ measurements were taken and the samples were serially diluted and plated onto either Luria-Bertani Agar for *S. aureus* or GYM Streptomyces agar[4] for *R. opacus* for viable cell determination. The plates were incubated for 16 hr at 37 °C for *S. aureus* and for 48 hr at 25 °C for *R. opacus* prior to counting the number of colony forming units (CFU). Viable cell count results are given in $CFU.ml^{-1}$. The gradients of the calibration curves in Figure S8 showed that OD unit (at 600 nm) of 1.0 is equivalent to approximately $7x10^8$ $CFU.ml^{-1}$ *S. aureus* cells, and $4x10^7$ $CFU.ml^{-1}$ *R. opacus* cells.

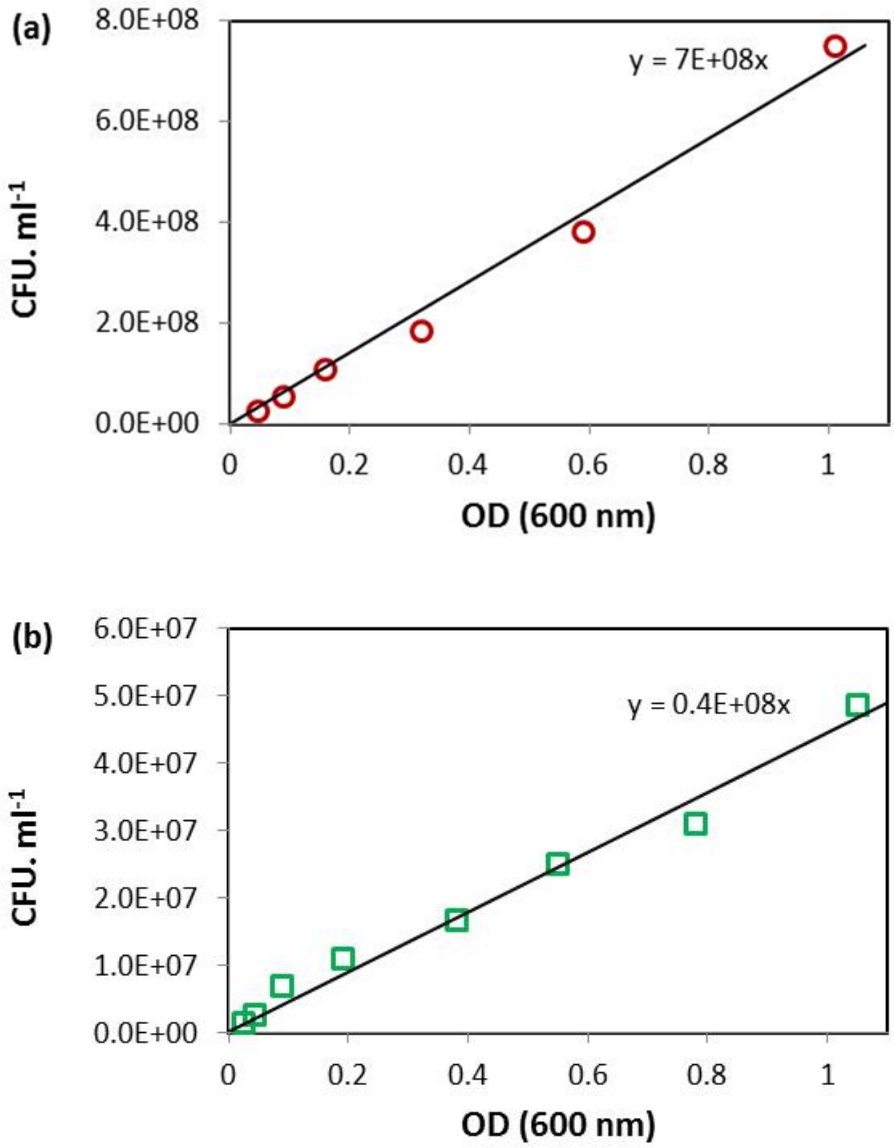

Figure S8. Calibration curves relating OD measurements (600 nm) versus viable cell count in CFU.ml^{-1} for (a) *S. aureus*, and (b) *R. opacus* cells. The slopes of the linear calibration curves are also given in each figure.

S9. Bacterial Growth Models

Cell growth rates for microbial cells are defined according to the general equation.[6]

$$\frac{dX}{dt} = \mu X \qquad (1)$$

where X is the amount of bacterial concentration at time t, and μ is the specific growth rate.

Due to the more pronounced lag phase within the GO wrapped cells, *logistic growth model* was applied for modelling the overall bacterial growth in all three phases (i.e. lag, exponential, stationary). Specific growth rate (μ) of the logistic model was defined[6] with equation (2) below, where k_c is the apparent growth rate and X_m is the maximum cell concentration:

$$\mu = k_c\left(1 - \frac{X}{X_m}\right) \qquad (2)$$

Rearranging the former equation after inserting into equation (1), and integrating between boundary conditions of X cell concentration at time t and and X_o initial cell concentration at time 0 yields the following:[6]

$$X = \frac{X_m}{1 + \left(\frac{X_m}{X_0} - 1\right).\ e^{-k_c.t}} \qquad (3)$$

The model parameters were calculated by a computer program (CurveExpert 1.4), with a logistic model equation design in cordial to the following logistic function. The logistic model constants are a= X_m, b= (X_m/X_0)-1, and c = k_c.

$$y = \frac{a}{1 + b.\ e^{-c.x}} \qquad (4)$$

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