Supplementary Information

Continuous flow vortex fluidic synthesis of silica xerogel as a delivery vehicle for curcumin

Chee Ling Tong[a], Uwe H. Stroeher[b], Melissa H. Brown[b], and Colin L. Raston[a*]

[a] Flinders Centre for NanoScale Science and Technology, School of Chemical and Physical Sciences, Flinders University, Bedford Park, SA 5042, Australia.

[b] School of Biological Sciences, Flinders University, Bedford Park, SA 5042, Australia.

*Corresponding author: Email colin.raston@flinders.edu.au

Experimental

Preparation of curcumin nanoparticles

Curcumin 108 mg was dissolved in 7 mL of ethanol. This solution was then fed into vortex fluidic device (VFD) at ~ 0.6 mL/min though feed jet 1 with Milli-Q water fed through a second feed jet at volumetric flow rate of 1.5 mL/min, while the 18 mm internal diameter borosilicate glass tube (NMR tube) was spun at 5000 rpm at a tilt angle of 45 ˚. The product was collected and then placed in a Rotavac for 5 min to remove the ethanol. The curcumin nanoparticles are well dispersed in water and ready to be used in the silica hydrogel synthesis.

Preparation of silica hydrogel

Silica hydrogel was prepared using the VFD under continuous flow. Tetramethyl orthosilicate (TMOS, Sigma Aldrich) was used without purification and was fed through one feed jet and Milli-Q water fed though another feed jet, with the flow rate in the range 0.8 – 1. 2 mL/min. The molar ratio of H_2O/Si for final sample was adjusted by modulating the volumetric flow rate of both feed jets. The gel samples were subsequently dried in an oven at 110 ˚C overnight and crushed into powder using a mortar and pestle, for further characterizations.

Preparation of curcumin loaded silica hydrogel

This followed a similarly procedure as used for the synthesis of the above silica hydrogel, except the Milli-Q water was replaced by the as formed freshly prepared suspension of curcumin particles. The molar ratio of H_2O/Si in the final product was fixed at 8:1. The final gel sample was dried using a Rotavac, with bath temperature set at 60 ˚C, for 30 min and the solid then converted to a powder using a mortar and pestle. These conditions minimize the likelihood of degradation of curcumin.

Preparation of agar for bacteria growth inhibition test

After removal of the ethanol, the as prepared suspension of curcumin was diluted with Milli-Q water in 15 mL tubes at concentrations from 209 $\mu g.mL^{-1}$ to 1047 $\mu g.mL^{-1}$. Mueller Hinton agar was then added to give a final volume of 10 mL.

Silica powder and powdered curcumin/SiO_2 were added to 15 mL tubes and Mueller Hinton agar was added and mixed to give a final volume of 10 mL. The final curcumin concentration for the curcumin/SiO_2 sample was adjusted to range from 60

μg.mL^{-1} to 300 μg.mL^{-1}. The agar plates were allowed to set and dried for 30 mins at room temperature prior to use.

Characterization techniques

Nitrogen adsorption-desorption studies were performed using a Micromeritics TriStar™ II 3020 system. The samples were outgassed at 110 °C for 16 hours prior to gas adsorption which was carried out at 77 K. The specific surface area, S_{BET}, was determined from the linear part of the Brunauer-Emmett-Teller (BET) equation (P/P_o = 0.05 – 0.30). The pore size distribution and pore volume were evaluated using the Barrett–Joyner–Halenda (BJH) method. The total pore volume reported was taken from the amount of gas adsorbed at a relative pressure (P/P_o) at 0.996.

Fourier Transform Infra-Red (FTIR) spectra were recorded on a Nicolet Nexus 870 FT-IR, equipped with a Thermo Scientific ATR-IR 'Smart Orbit Attachment'. The data was collected at 4 cm^{-1} resolution with 64 scans at room temperature.

^{29}Si MAS NMR was performed using a Bruker 400 instrument, single pulse at 5000 Hz rotating speed to determine the poly-condensation degree of the silica.

Dynamic light scattering (DLS) measurements were collected using a Malvern Instrument 'High Performance Particle Sizer' model HPP5001. The measurements were performed at room temperature and 10 measurements were taken for each run for 3 runs in total. Correlation data was analyzed using the Malvern Instrument software.

Scanning electron microscopy (SEM) images of curcumin nanoparticles were obtained using a FEI Quanta 450 FEG Environmental Scanning Electron Microscope (ESEM), operating at an accelerating voltage of 5 kV with secondary electron detector under high vacuum. A 5 nm thickness of platinum coating was applied onto the sample surface using Quorumtech K575X sputter coater prior to imaging.

The concentration of curcumin in the final samples was determined using two methods, involving the use of a Varian Cary Eclipse Fluorimeter and Simultaneous Thermal Analyzer (STA), to compare the consistency. For fluorimeter studies, the excitation wavelength was set at 510 nm and emission wavelength at 555 nm. Standard solutions of known curcumin concentration were prepared by dissolving curcumin in ethanol. A calibration curve was plotted by linear fit correlation and the final concentration of curcumin in the sample was determined using the calibration fit equation. STA combines thermo-gravimetric analysis (TGA) and differential scanning calorimetry (DSC) for analysis of the thermal properties of inorganic and organic materials. Sample was heated under nitrogen from 30 to 600 ˚C, with the temperature increment rate at 10 ˚C/min.

Characterization data

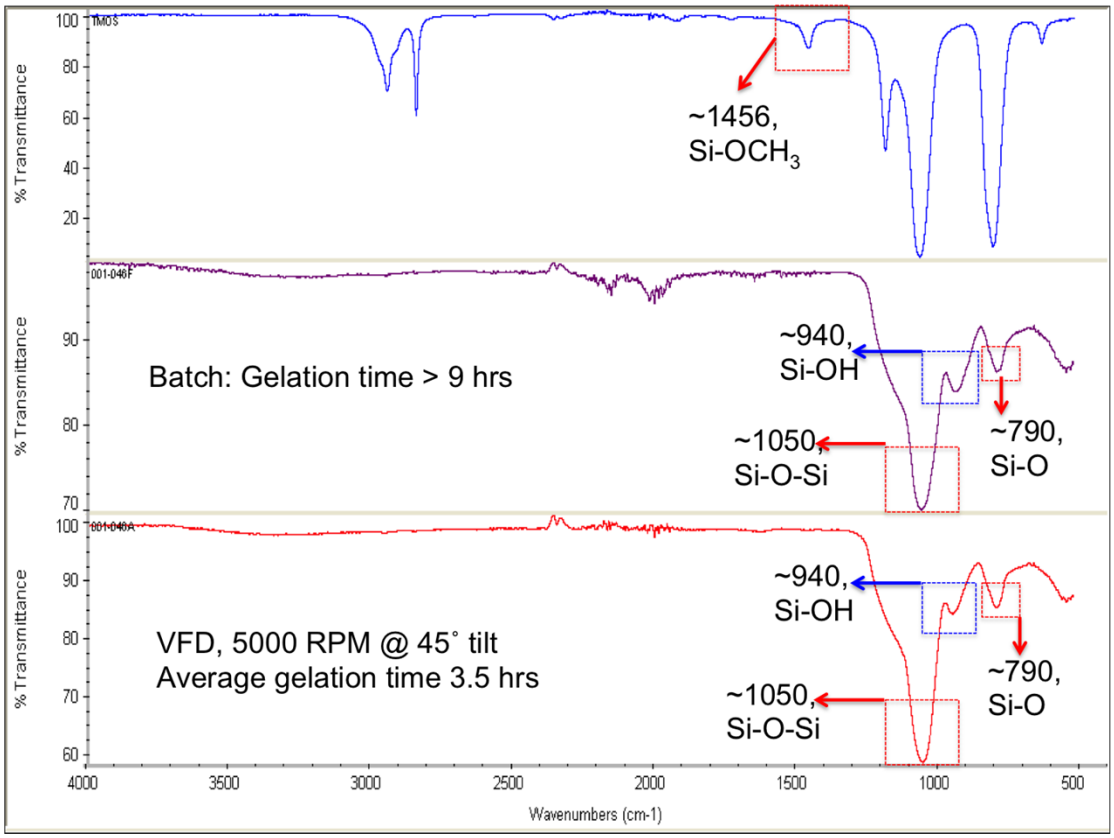

Figure S1. FTIR spectra of TMOS, batch processed silica xerogel, and VFD processed silica xerogel.

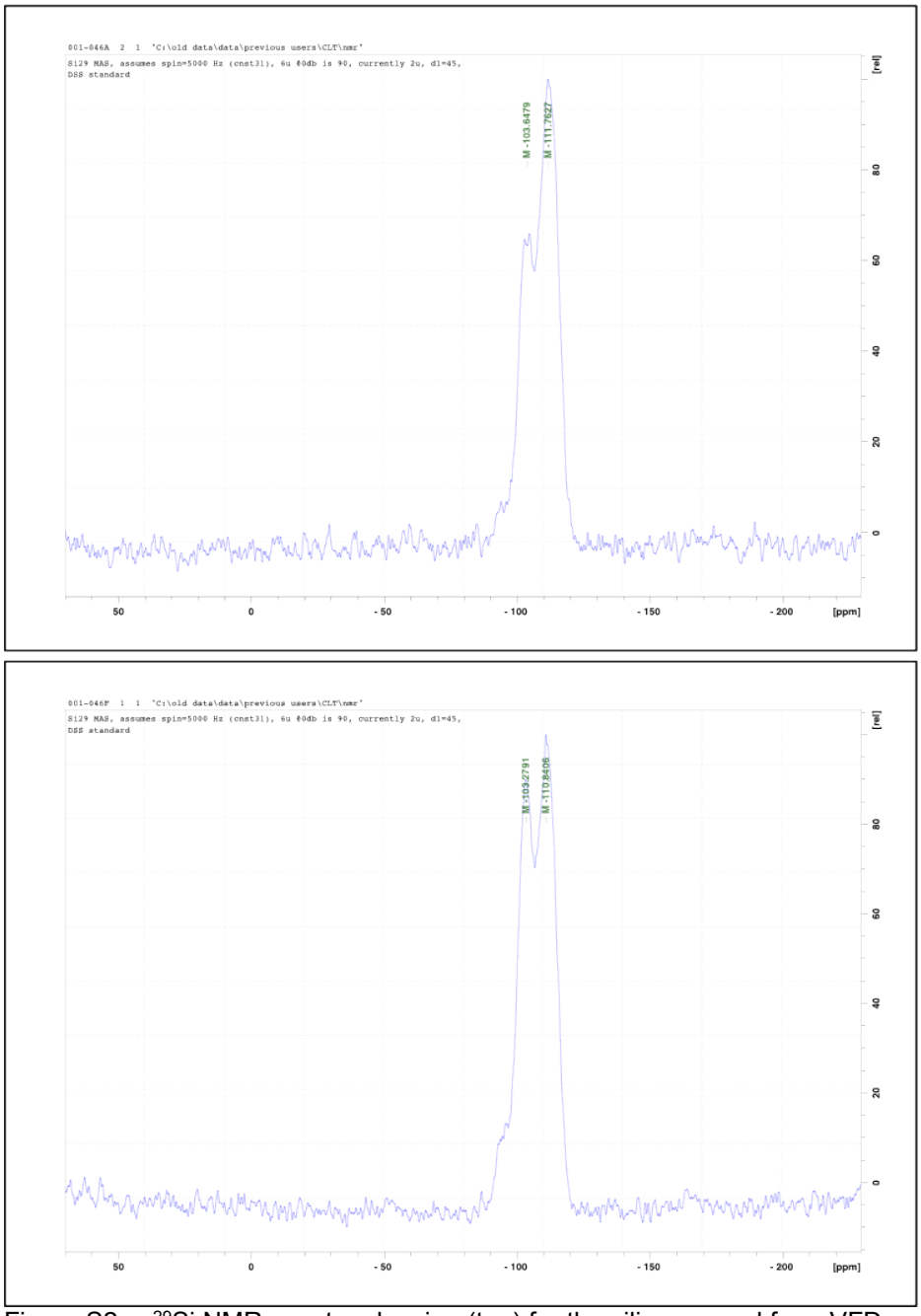

Figure S2. ^{29}Si NMR spectra showing (top) for the silica xerogel from VFD-processing at 5000 rpm, at a 45˚ tilt angle, and (bottom) batch-processed silica xerogel.

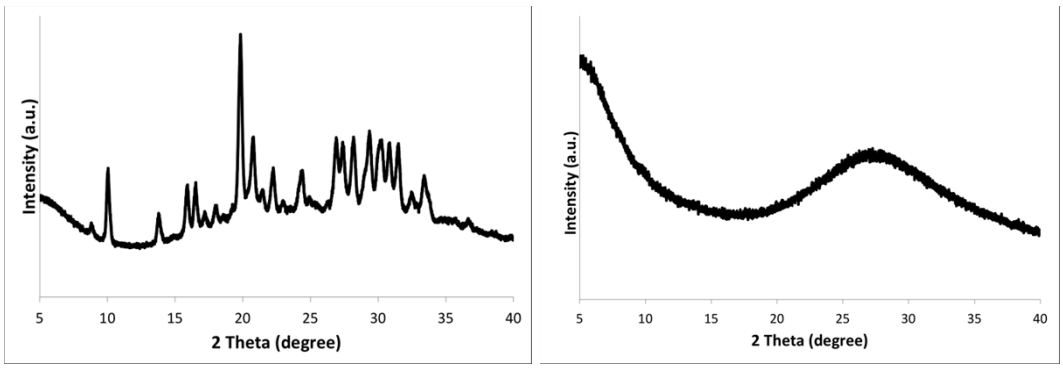

Figure S3. X-ray diffraction pattern of (left) crystalline curcumin and (right) silica xerogel loaded with curcumin.

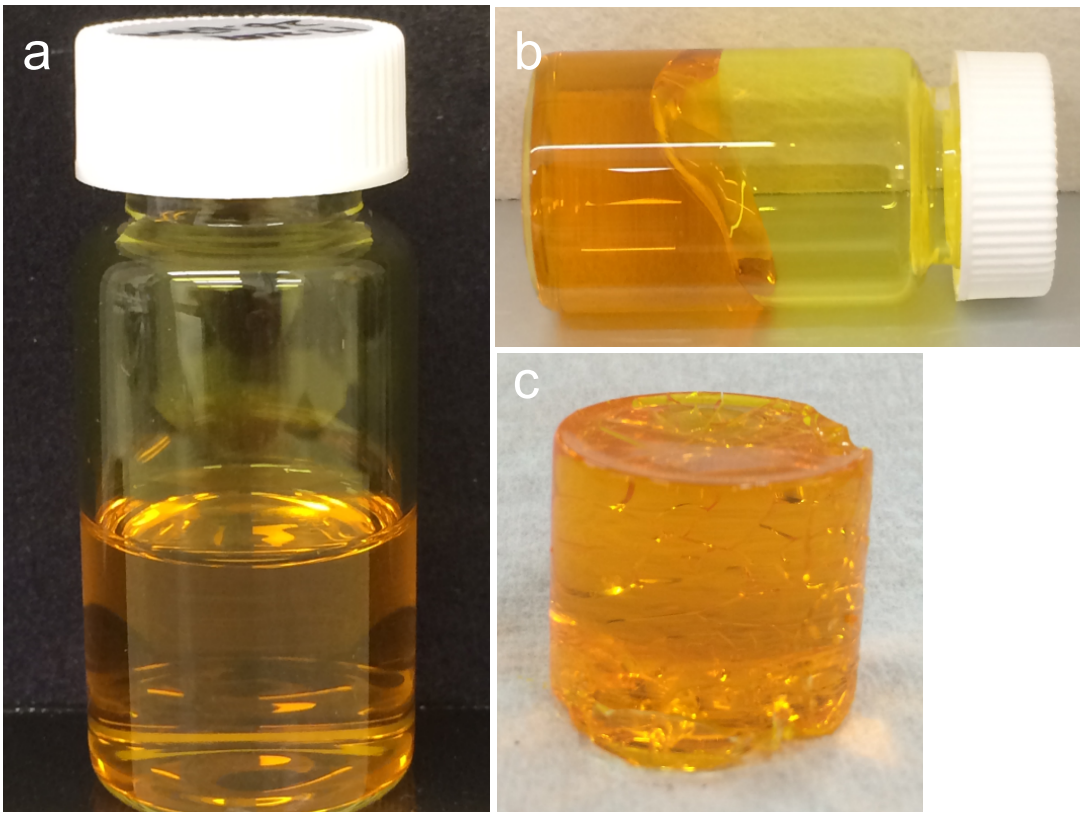

Figure S4. Curcumin loaded silica hydrogel (a) initial appearance from the VFD; (b) partial gelation; and (c) curcumin/SiO_2 gel. The sample was prepared with the VFD operating at 5000 RPM at 45˚ tilt.

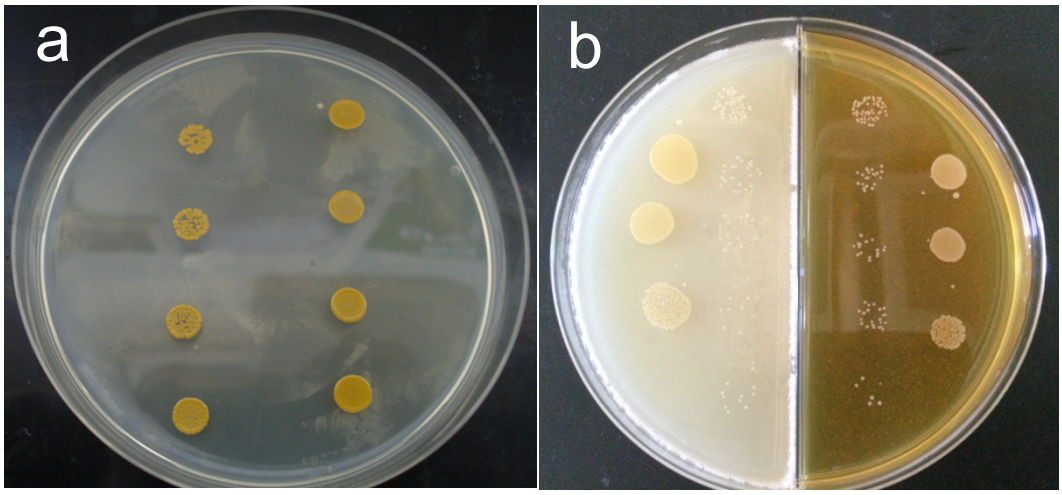

Figure S5. Bacterial growth inhibition test; (a) growth on agar only, (b) growth with 1 g of silica xerogel (left), and a suspension of curcumin particles, with the curcumin at 194 μg.mL^{-1} (right)

Table S1. *Staphylococcus aureus* bacteria growth inhibition test comparison.

Sample: Curcumin/SiO_2 composite		Sample: Suspension of curcumin particles	
Curcumin concentration $\mu g.mL^{-1}$	Inhibition*	Curcumin concentration $\mu g.mL^{-1}$	Inhibition*
60	No	209	No
120	Mild	419	No
180	6/8	628	2/8
240	7/8	838	4/8
300	Full	1047	Full

* The inhibition numbers show in term of fraction, referring to the successful growth inhibition number out of 8 dilutions.

Curcumin content using fluorescence spectroscopy:

A series of known concentration of curcumin solutions in ethanol were prepared and used to obtain a calibration curve, giving a linear equation:

$y = 215.83\ x + 3.1777$ with linear regression $R^2 = 0.9521$; where x is the curcumin concentration in $mg.mL^{-1}$.

The curcumin was extracted from 0.3054 g of agar/composite curcumin/SiO_2 in ethanol, for then recording the fluorescence spectra, affording a concentration of curcumin at 71 $\mu g.mL^{-1}$.

The weight of agar nutrient solution was 9.0 g and 1.25 g curcumin/SiO_2 composite was added:

$$\%\ Composite\ silica\ with\ curcumin = \frac{1.25}{10.25} \times 100\% = 12.20\%$$

Thus, total weight of composite silica with curcumin in 0.3054 g of gel is:

$$0.3054\ g \times 12.20\ \% = 37.26\ mg$$

Therefore, weight percent of curcumin in the composite sample is:

$$\frac{0.071}{37.26} \times 100\% = 0.19\ \%_{\text{w/w}}$$

Graphs below provide another method for estimating the curcumin content. This STA method affords a curcumin content of 0.24% w/w.

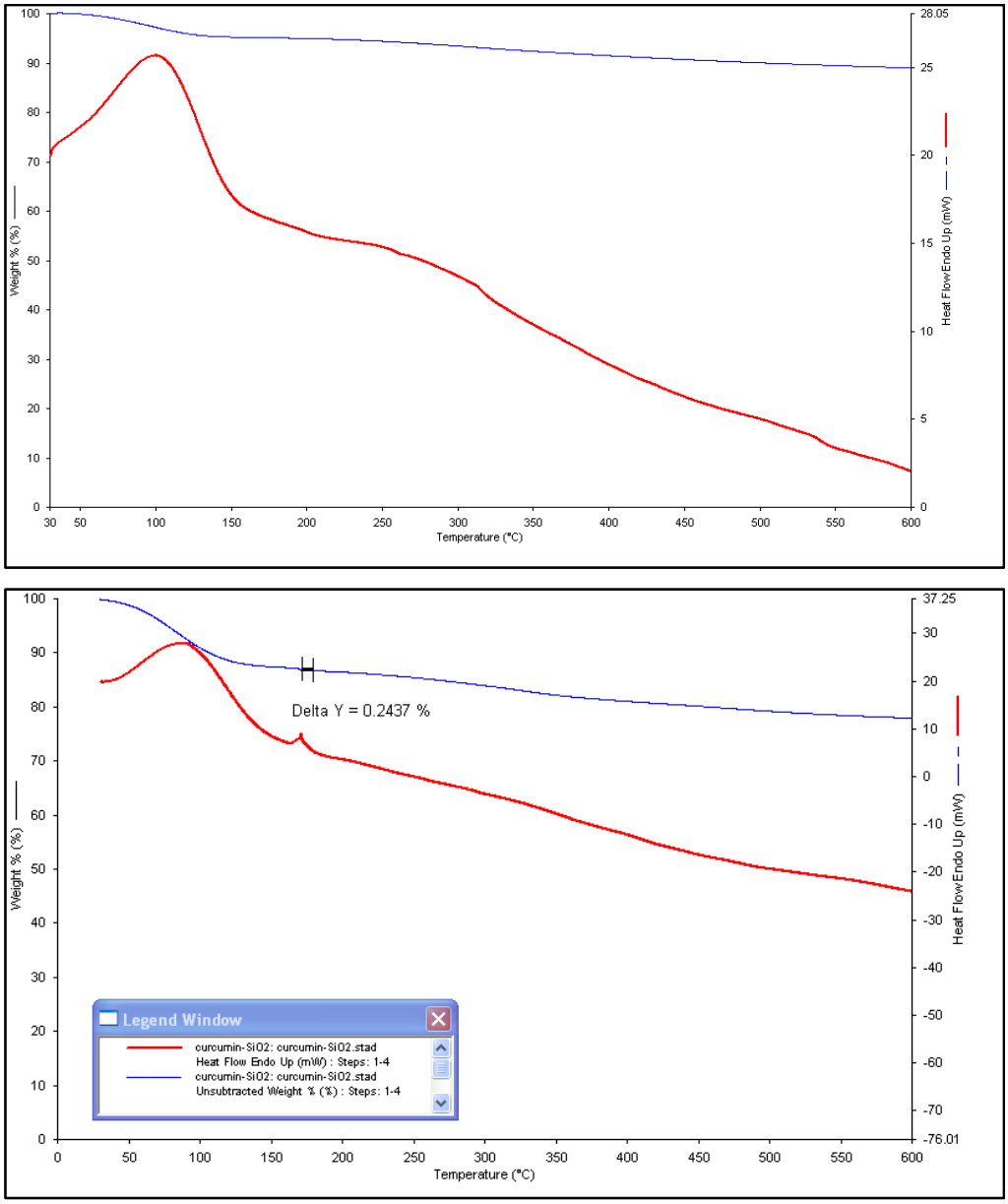

Figure S6. STA plot of silica xerogel devoid of curcumin showing a small weight loss associated with surface hydroxyl groups (top), an endotherm peak at 172 – 179 ˚C , consistent with the decomposition of curcumin in the composite material (bottom).

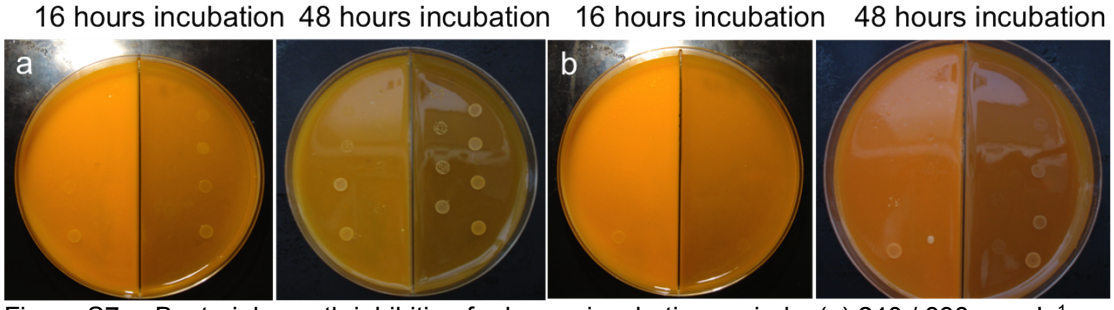

Figure S7. Bacterial growth inhibition for longer incubation periods; (a) 240 / 838 μg.mL^{-1}, and (b) 300 /1047 μg.mL^{-1}.