Supplementary Material (ESI) for Chemical Communications

Sol-gel encapsulation of cells is not limited to silica: bacteria long-term viability in alumina matrices

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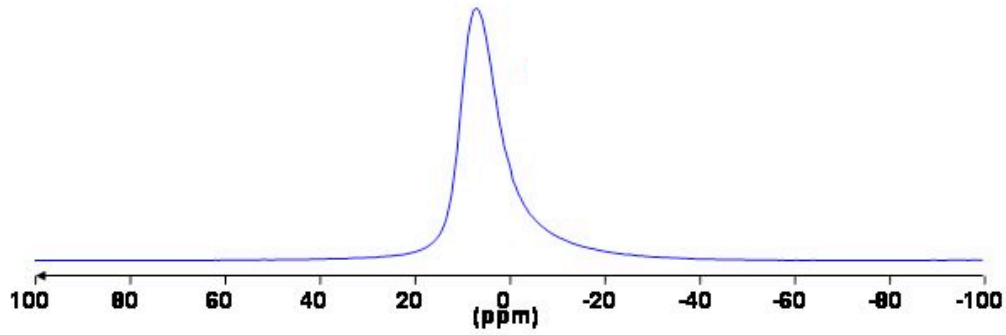
Electronic Supplementary Informations

ESI-1 : Solid state ^{27}Al MAS NMR spectra of the boehmite gel
ESI-2 : Detailed experimental procedures

ESI-1 : Solid state ^{27}Al MAS NMR spectra of the boehmite gel



ESI-2 : Detailed experimental procedures

Bacterial strain and growth conditions

Stock cultures of cells were prepared from cultures stored at -80 °C in LB broth supplemented with glycerol. When needed, cultures of bacteria in LB broth at 37 °C were prepared overnight. An inoculum (0.1 mL) was added to the minimum phosphate broth (minimum medium) at pH 7 (50 mL) in the presence of glucose (20 mM) as a growth substrate in a flask (250 mL) and grown at 37 °C under stirring (200 rpm). After 3 h of incubation, corresponding to the mid-exponential phase of growth, the culture was harvested by centrifugation at 6000 rpm for 15 min at 9 °C. The pellet was washed twice with PB (100 mM), and diluted to reach a concentration of 10^9 cells mL^{-1} in PB (working cell suspension, WCS) or in 10% w/w glycerol PB (gly-WCS).

Growth of bacteria was studied in a minimum medium containing glycerol (10%w/w) MM. In a typical experiment, a bacteria inoculum (100 μL) was added to the culture medium MM (50 mL) in the presence of glucose (20 mM) as a growth substrate. In order to study their effect, boehmite particles, in different concentration (50, 5 and 0.5 $g.L^{-1}$), were added. Cells were incubated at 37 °C under stirring. The growth of bacteria was followed by turbidity measurements; the optical density (OD) of suspension aliquots recorded at λ = 600 nm is known to be proportional to bacteria concentration.

Preparation of boehmite sol

Boehmite sols were produced by dissolving aluminium chloride salt (Acros Organics) in deionized water, and adjusting the pH at 8 by the addition of sodium hydroxide. The final aluminium concentration is 0.7 $mol.L^{-1}$. The solution was then processed hydrothermally in an autoclave at 95 °C for one week. The resulting precipitate was recovered by centrifugation and washed with pure water until no chloride was detected by adding $Ag(NO)_3$. A Boehmite sol (BS) was prepared by adding 1.5 g of boehmite precipitate in 1 mL of pure water.

Encapsulation of cells and references

The gel was formed by adding PB or WCS solution (1 mL) in the Boehmite sol (BS) previously prepared. The mixture was homogenized under gentle stirring (300 rpm). Gelation occurred instantaneously at room temperature. Wet gels were aged for 1 hour and 1, 15 or 30 days at 20 °C in the mother solution in a closed flask

Viability measurements

Wet gels were crushed, vigorously stirred with phosphate buffer (3 mL) for 1 hour after one, two and four weeks. A series of 10-fold dilutions in phosphate buffer of WCS or resuspended gels (0.1 ml) diluted in PB were surface-plated in triplicate on LB-agar. Plates were then incubated at 37 °C for 24 h. The colony forming units count after one day is taken as a 100% reference.

Gel characterization

Cells containing aerogels were also obtained by supercritical drying in CO_2 after fixing the boehmite gel in 2.5% glutaraldehyde and dehydration through ethanol baths (30%, 50%, 70%, 80%, 90%, 95% and 100%).

Then the samples were coated with gold in a Balzers Union SCD 40 sputter-coater and studied by scanning electron microscopy (SEM) using a Cambridge Stereoscan 120 instrument at an accelerating voltage of 10 kV.

The aged wet boehmite gels containing cells were fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetraoxide. The samples were then dehydrated through successive ethanol baths (50%, 70%, 95%, 100%) prior to inclusion in araldite. An ultra microtome (Ultracut Reichert Jung) was used to section the block into 70 nm thin sections that were stained with phosphotungstic acid for electron microscopy. Observation of ultra thin sections by transmission electron microscopy was performed on a JEOL JSM-5510 LV at an accelerating voltage of 100 kV

The porosity of aerogels was measured by nitrogen sorption experiments performed at 77 K on a Micromeritics 2010 sorptometer. Prior to analysis, samples were first degassed at 60 °C under a 3 μm Hg pressure. Specific surface areas S_{BET} were determined by the Brunauer–Emmett–Teller (BET) method in the relative pressure range of 0.05–0.3

^{27}Al solid-state MAS NMR spectra of xerogels, without bacteria, were recorded on a Bruker MSL 400 spectrometer operating at 104.28 MHz with a recycle delay of 0.5 s with a pulse length corresponding to a $\pi/12$ angle . Xerogels were spun at 14 kHz using 4 mm rotors; 800 scans were accumulated.