Electronic Supplementary Information

Hydrophobicity-Responsive Engineered Mesoporous Silica Nanoparticles: Application to the Delivery of Essential Nutrients to Bacteria Combating Oil Spills

Nora Corvini, Mohamed El Idrissi, Eleni Dimitriadou, Philippe F.-X. Corvini and Patrick Shahgaldian

University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Hofackerstrasse 30, CH-4132 Muttenz, Switzerland

Table of content

1. General

Solvents and chemicals were purchased from Merck (Switzerland) and silanes from ABCR (Germany) and used without further purification. Water was purified with a Millipore Synergy purification system (resistivity ≥ 18 MΩ cm). UV-vis spectroscopy experiments were carried out using a Synergy™ H1 hybrid multi-mode microplate reader (Biotek, Switzerland). All measurements were performed at 25°C using Greiner Bio-one 96 well UV-Star microplates.

2. Experimental procedures

2.1. Synthesis

Mesoporous silica nanoparticles (mSNPs) were produced as previously described.[1]
mSNPs (20 g) were dried in a round bottom flask at 80°C under vacuum. Solutions of urea and potassium phosphate (K_2HPO_4) of increasing concentrations (*cf.* manuscript) were prepared and added to the flask (400 mL for 20 g) containing the mSNPs and maintained under magnetic stirring for 12 h. The particles were filtrated and dried under vacuum at room temperature for 12 h. The mSNPs were then suspended in toluene (200 mL) in a round bottom flask. A solution of trichloro-octadecylsilane (3 mL) was then added to the suspension. The mixture was stirred for 20 h at 20°C. The particles were susbsequently filtrated and washed with heptane and dried at 30°C under vacuum.

2.2. Scanning electron microscopy

Scanning electron micrographs were acquired using a Zeiss SUPRA® 40VP field-emission scanning electron microscope. A volume of 5 µL of each sample was spread on freshly cleaved mica sheets, dried under ambient conditions and sputter-coated with a gold-platinum alloy for 30 s at 15 mA (SC7620 Sputter coater). Micrographs were acquired using the InLens mode with an accelerating voltage of 10 kV.

2.3. Transmission electron microscopy

Transmission electron microscope measurements were performed on a Zeiss EM900 microscope equipped with an Olympus Megaview III CCD camera. A volume of 10 µL of suspension was spread on TEM copper grid and left to dry under ambient conditions prior to characterization.

2.4. Infrared spectroscopy

Fourier transform infrared spectra were measured in attenuated total reflection (ATR) mode using a single reflection diamond ATR device (Golden Gate) and a Varian 670-IR spectrometer, spectra were recorded between 500 and 4000 cm^{-1}.

2.5. ζ-potential measurements

ζ-potential measurements were carried out on a Malvern ®Zetasizer nano-ZS instrument with Malvern® DTS 1070 cells after equilibration for 2 minutes at 25°C.

2.6. Urea concentration assay

Samples of 100 µL were recovered from the supernatant and added to water (900 µL). Subsquently were sequentially added diacethylmonoxime (50 µL), thiosemicarbazide (8 µL) and $Fe_2(SO_4)$ (8 µL) and H_2SO_4 (667 µL).[3] The mixture was heated to 85°C for 30 min and then cooled down by immersing the assay tubes in water at 20°C for 10 min. Samples of 200 µL were added to the well plate and absorbance was measured at 520 nm; measured values were compared to a standard curve obtained with urea.

2.7. Triggered release study

Phosphate release experiments were carried out using a commercial colorimetric kit (Merck, Switzerland). mSNPs equipped with the C_{18} hydrophobic gate system and loaded with phosphate were suspended in 1:1 water/heptane mixture (200 mL) and submitted to vigorous magnetic stirring. In order to measure the phosphate release, for every measurement, the magnetic stirring was stopped and a volume of 1 mL of water was collected; phosphate concentration was measured using a commercial colorimetric kit following the manufacturer protocol.

2.8. Biodegradation assays

Assays were carried using *Marinobacter hydrocarbonoclasticus* KS-ANU5 in order to test the efficiency of stimulating the microbial degradation of crude oil through the amendment of N and P with mSNPs. Experiments were carried out in 100 mL Erlenmeyer flasks filled with 20 mL of Artificial seawater medium,[2] and 0.1g of crude oil DUC (Danish Underground Consortium (0.5% w/v). Artificial seawater was inoculated using *Marinobacter* strain with a final biomass of 5.3 10^7 CFU/mL with the addition or not of mSNPs (representing 100 mg L^{-1} and 10 mg L^{-1} of N and P, respectively). Additional abiotic controls were also carried out. For each sampling time, the corresponding incubation vessels were sacrificed and remaining hydrocarbons were extracted using liquid-liquid extraction with *n*-hexane. Prior to extraction, 1-chlorooctane (50 µL/L) was added to the samples as internal standard in order to quantify extraction yields. In order to break the oil droplets, samples were treated in ultrasonic bath for 3 min. The recovered extracts were poured over silica and glass wool columns packed in Pasteur pipettes in order to remove the non-soluble fraction and to clear the samples. Samples were analyzed by means of gas chromatography-mass spectrometry (GC-MS) using a C_8-C_{40} alkanes calibration standard (Supelco). The extent of hydrocarbon degradation (remaining hydrocarbons) was determined using GC-MS for the analyses of the organic extracts.

2.9. Gass chromatography

GC (Agilent) was performed using Zebron ZB-5HT Inferno – Phenomenex columm (30 m x 250 µm x 0.25 µm). Helium was used as carrier gaz. The injector port and the transfer line were operated at 280°C and the temperature gradient was programmed at 60°C for 0.5 min then 7.5°C/min to 150°C for 0 min, then 10°C/min to 250°C for 0 min and then 15°C to 340°C for 8 min. The injection volume was 1 µL. Spectra were obtained by electron impact ionization (70 ev). The quantification of oil components during incubation was performed for seven alkanes, namely dodecane, tridecane, tetradecane, pentadecane, octadecane, nonadecane, and tetracosane as well as for four aromatic hydrocarbons, i.e. benzene, naphthalene anthracene and phenanthrene.

3. Results

3.1. ζ-potential measurements

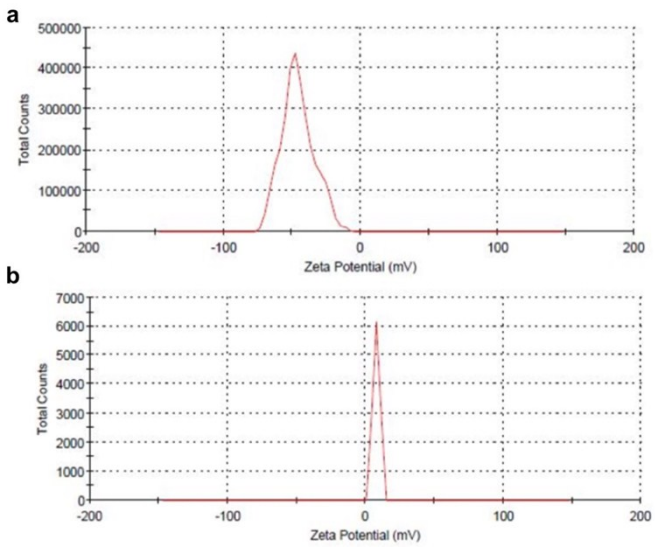

Fig. S1 Zeta potential of mSNPs measured before (**a**) and after (**b**) the implementation of the gate system

3.2. Degradation of linear alkanes

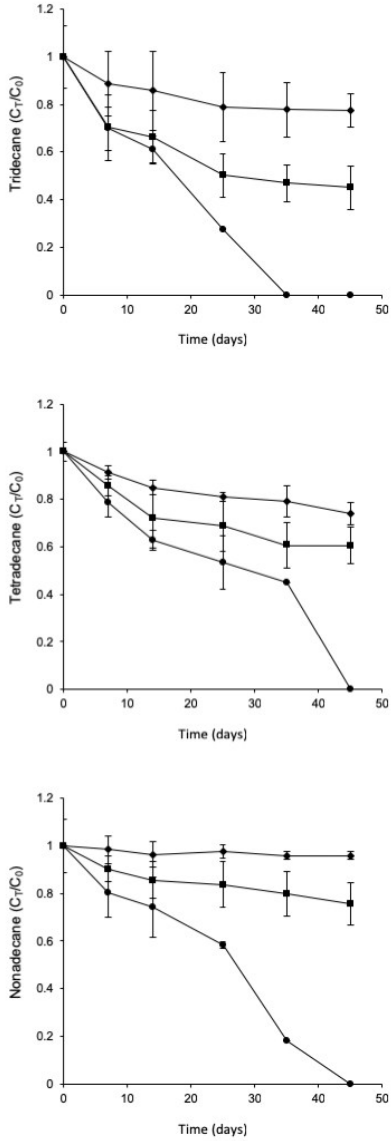

Fig. S2 Degradation of alkanes in abiotic controls (•) and in inoculated samples with (•) or without (□) gated particles. *y* axes represent the ratio of the concentration remaining at sampling time *t* rationalised using the initial concentration of the tested compound. Error bars represent standard deviations (n=3).

3.3. FTIR characterisation

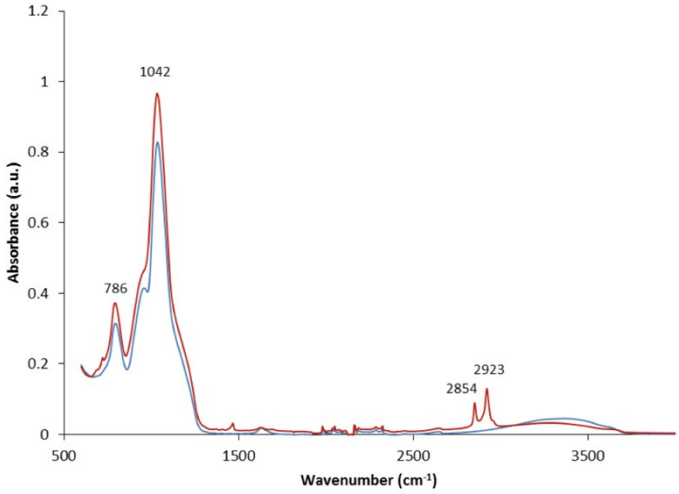

Fig. S3 FTIR characterization of the silanisation reaction, characteristics peaks of the alkyl chains (C-H stretch at 2854 cm^{-1} and 2923 cm^{-1}) of the C_{18} chains are visible on the modified mSNPs (red) and absent on the unmodified ones (blue).

4. References

1. C.-Yu Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija, V. S.-Y. Lin, *J. Am. Chem. Soc.*, 2003, **125**, 4451-4459.
2. J. A. Berges, D. J. Franklin, P. J. Harisson, *J. Phycol.*, 2001, **37**, 1138-1145.