# Multifunctional porous materials through ferrofluids

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This communication introduces an innovative, simple and easy procedure to develop advanced multifunctional materials derived from the treatment of diverse porous solids with ferrofluids at moderate temperature, which confer superparamagnetic properties to the final system allowing diverse advanced applications.[1] These new systems, regarded as *supported* or *dry ferrofluids*, can show additional functionalities afforded by the solid support. The superparamagnetic porous solids result from the assembling of non-aqueous ferrofluids constituted by magnetite nanoparticles bonded to oleic acid molecules that can be anchored to the solid surface through its hydroxyl groups. The most significant feature of this approach is related to the preservation of the characteristic adsorbent properties of pristine microporous solids, such as silica, clay minerals, zeolites or activated carbons. Their superparamagnetic behaviour allows their controlled separation after the adsorbate uptake by application of an external magnetic field without loss of the immobilized nanoparticles (NPs) as showed in video S1. This is especially relevant in technological, environmental and biomedical applications where it is necessary to adsorb and remove certain species from aqueous media avoiding

conventional separation processes such as filtration or centrifugation. Moreover, this methodology can be relevant for the development of other multifunctional materials provided with superparamagnetic behaviour as accounted below.

It is well known that the most common type of ferrofluids is based on magnetite ($Fe_3O_4$) NPs, due to the versatility of preparation methods and chemical modification of the NPs that allows the possibility to use either aqueous or organic media as solvents.[2-5] The already reported approaches on immobilization of magnetite NPs on porous solids such as zeolites and clays are mainly based on the *in situ* generation of NPs from different precursors (one-pot procedure)[6,7] or by impregnation of the solid with modified NPs previously synthesized (post-synthesis procedure).[2,3] In this last case, NPs are usually incorporated together with other additives in aqueous media, which may result in NPs aggregation and the consequent loss of superparamagnetism.[8] The new procedure here reported and recently patented[1] is based on the impregnation of porous solids using a non-aqueous ferrofluid that allows a homogenous distribution of anchored oleic acid-modified magnetite NPs on the solids surface, maintaining their adsorbent properties together with superparamagnetic behaviour of the assembled NPs at room temperature. Diverse microporous solids such as silica, clays, zeolites or activated carbon were treated at room temperature with ferrofluids prepared from dispersions of oleic acid-magnetite NPs in hydrocarbons (e.g., n-heptane). The modified magnetite NPs are disaggregated in the ferrofluid exhibiting superparamagnetic behaviour (98 emu/g of total material, i.e. oleic acid-magnetite NPs, in which the oleic acid content determined from CHN chemical analysis is approximately 16% of the total mass) and, in a similar way, they remain disaggregated after assembling to porous solids conferring their magnetic properties to the new materials. Considering for instance the sepiolite clay mineral (Fig. 1a), a microporous magnesium silicate of microfibrous morphology,[9,10] it

has been treated with increasing amounts of oleic acid-magnetite ferrofluid in n–heptane that allowed to obtain sepiolite-NPs in the range of 5-80% in weight. After complete evaporation of the organic solvent, the oleic acid-magnetite NPs remain covering the surface of the silicate as observed by TEM (Fig.1c). In addition to the presence of bands ascribed to oleate groups, the FTIR spectra (Fig. S1) indicates a strong decrease in intensity of the stretching O-H bands of Si-OH groups (at ca. 3720 cm$^{-1}$) covering the sepiolite surface.[11] This behaviour suggests the involvement of these surface groups in the interaction with the magnetite NPs, in a similar way to that observed in anatase NPs anchored on sepiolite surface.[12] XRD patterns indicate the preservation of magnetite and sepiolite structures (Fig. S2). The widths of the reflections are compatible with a NPs average size that is in the order of magnitude of 10 nm, in accordance to TEM observations (Fig. 1b). These observations point out to a tentative mechanism of ligands exchange in which the NPs liberate oleic acid molecules (that can remain adsorbed in the microporous solid) and the iron oxide becoming in interaction with the silanol surface groups of sepiolite (Scheme 1).

It is possible to eliminate the oleic acid assembled to the resulting materials by calcination at around 500 ºC (air flux), revealed by the disappearance of its IR bands in the spectra, the magnetite NPs remaining anchored to the silicate. Due to the oxidative conditions of those thermal treatments there is a partial oxidation of magnetite to maghemite visualized by the reddish coloration of the starting black sample, which is corroborated by the observed changes in the magnetic behaviour (*vide infra*).

Magnetic characterization of the sepiolite after assembly of the oleic acid-magnetite NPs samples using Vibrating Sample Magnetometry (VSM) at room temperature shows hysteresis loops typical of superparamagnetic behaviour, i.e. an elevated magnetic susceptibility with absence of coercive field (Fig. 2a). The corresponding saturation

magnetization values are directly proportional to the NPs content in the samples (Fig. 2b and 2c). Thermal treatment for the complete removal of the oleic acid results in a slight decrease of the saturation magnetization value, behaviour that can be tentatively attributed to a partial oxidation of magnetite to maghemite as indicated above.

It has been reported that magnetic nanoparticles, such as Fe and $\alpha$-$Fe_2O_3$, can be incorporated in sepiolite using a synthetic approach that implies acid and thermal treatments at relatively high temperatures.[7,13] The resulting materials show ferromagnetic behaviour that has been profited for the preparation of magnetic humidity sensors[7] and to decorate ceramic materials.[14] In the materials here reported, sepiolite does not suffer any drastic treatment, preserving its adsorbent properties, and the magnetic behaviour is superparamagnetic, i.e., it is only magnetic in the presence of a magnetic field. Therefore, the sepiolite-NPs materials here prepared in this work can give rise to multifunctional materials of interest for different types of applications.

The coverage of the sepiolite surface by the NPs produces a reduction of the specific surface area (BET, $N_2$) due to the micropores blockage, avoiding the accessibility of nitrogen molecules during the corresponding measurements. The aspect of the $N_2$ isotherms of sepiolite-NPs samples (not shown) is quite similar to that of pristine sepiolite (isotherm type I/II with hysteresis loop of type H3 according to the IUPAC classification),[15] but the micropore volume is drastically reduced upon incorporation of the NPs, probably due to the adsorption of oleic acid molecules into the micropores defined by the intracrystalline cavities (tunnels) of nanometric dimensions in sepiolite. [11] It should be taken into account that the non-linear conformation of oleic acid molecules avoids their complete penetration into the tunnels. Despite the decrease of the specific surface area (Fig. 3a C) by the micropores blockage, the ability of sepiolite-NPs to maintain its adsorbent properties towards different type of species in water is

especially relevant. The samples are easily recovered after uptake with the help of a magnet. A cationic dye, methylene blue (MB), has been tested as model molecule to show that the porous solid acting as support of the ferrofluid maintains its sorption ability[16] and, after the uptake of MB, it can be completely removed without using the classical separation processes (filtration or centrifugation). The optimal efficiency of the system is achieved for samples prepared from 35-50% NPs in weight being able to adsorb at least 97% of MB in a $5\times10^{-4}$ M MB solution, which is close to the MB adsorption capacity of pristine sepiolite (> 99%). In these conditions, MB solutions $<10^{-3}$ M become completely transparent as indicated by the UV-Vis spectra (video S1). Similarly to sepiolite, other porous solids such as silica gel, smectite clays, zeolites and activated carbons also act as efficient supports of oleic acid-magnetite NPs ferrofluids, showing superparamagnetic behaviour (see for instance Fig. 2e). The porous solids show different compositions, morphologies and crystal structures but all of them are provided with surface OH groups and microporosity, which are the apparent requisites to act as efficient supports. Moreover, all of them exhibit elevated specific surface area and adsorption capacity and, therefore, once the superparamagnetic NPs are incorporated the resulting materials are provided with both properties: sorption capacity and paramagnetic behaviour. Fig. 3a and Fig. 3b show the specific surface area and the uptake behaviour of MB for some of these supports, respectively, before and after assembly to oleic acid-magnetite NPs in a 1:1 ratio.

It must be signalled that smectite clays and zeolites also exhibit ion-exchange ability that can be profited for (selective) adsorption of cations, which can be of great relevance for the easy and safe magnetic removal of heavy metal ions or radionuclides. Zeolites are often used as catalysts and therefore, the incorporation of the NPs can also be of interest to recover the catalyst from the reactor after the catalytic process. Moreover,

supports such as silica and sepiolite can be modified by incorporation of diverse functionalities mainly by covalent bonding through their Si-OH for diverse applications. All these features show the great potential interest of the reported approach to prepare multifunctional materials. To illustrate this potentiality we report here two additional examples consisting in the development of superparamagnetic hybrid buckypapers containing multi-walled carbon nanotubes (MWCNTs) and bioreactors with incorporated biomimetic lipid membranes.

*Hybrid buckypapers* based on sepiolite-MWCNTs assembly have been recently developed in our group.[17,18] These materials result from the ultrasonic-driven assembly of filamentous materials, sepiolite and carbon nanotubes (CNTs) in aqueous conditions, forming a tightly entangled mesh of both types of solids (Fig. 1d). Besides the clear advantages that arise from using CNTs in aqueous media, the materials obtained from this approach present excellent electronic conductivity along with the easily available chemistry, which is characteristic for this fibrous silicate. In the case of superparamagnetic materials such as the sepiolite-magnetite NPs in a 1:1 ratio here developed in this work, the inclusion of a carbonaceous electronic conducting phase is expected to be advantageous, as it configures a simple way to build materials applicable in Electromagnetic Interference (EMI) shielding.[19] The formation of a conductive percolating network is especially relevant in the case of microwave shielding.[20] In addition to the magnetic properties typical of the magnetite NPs (Fig. 2d), the electrical conductivity of the resulting hybrid buckypapers-magnetite NP films (containing 5% of MWCNTS in the superparamagnetic material) presents no variation whatsoever when compared to the electrical conductivity previously reported for the original *hybrid buckypapers* containing 5% of MWCNTs (around 1.6 S.cm$^{-1}$). The specific saturation magnetization for the hybrid buckypapers-NPs is around 35 emu.g$^{-1}$. When corrected to

the real content of magnetite NPs (47.5% from which one fifth part corresponds to oleic acid), the saturation magnetization becomes 91 $emu.g_{NP}^{-1}$ which is in good agreement with the initial magnetic properties determined for magnetite nanoparticles alone. In summary, the process of assembling magnetite modified sepiolite to MWCNTs does not imply a decrease in the specific saturation magnetization of the corresponding NPs and also the superparamagnetic behaviour of pristine NPs is maintained. This result suggests that this strategy of incorporation of a conductive matrix might be of great utility in the development of electric and magnetic multifunctional materials based on supported ferrofluids on sepiolite.

Finally, in order to assess the possibility to use the here introduced NPs-porous solid systems as carriers of enzymes for the development of superparamagnetic bioreactors, the membrane-bound enzyme cholesterol oxidase (COx) was chosen as a model for immobilization on sepiolite-magnetite NPs support. A key factor in the design of enzyme reactors is the preservation of the catalytic activity after immobilization and under process conditions, as well as throughout recovery and recycling.[21] The presence of an oleic acid monolayer on the sepiolite-magnetite NPs serves as an appropriate support for immobilization of a phospholipid (phosphatidylcholine, PC) monolayer, similarly to that previously reported for related systems.[22] The resulting mixed oleic acid/PC bilayer (Fig. S3) may accommodate the enzyme, as already performed on similar supported lipid interfaces.[23] The resulting superparamagnetic materials (Fig. 2f), loaded with ca. 50 µg/mg of COx, were tested in a configuration of batch reactor, in which the easy recovery of the catalysts after reaction is also a crucial factor. Thus, the superparamagnetic properties of the sepiolite-NPs can be profited for recovering the catalyst from reaction media when the enzymatic process is completed, just by applying a magnetic field, as reported for non-supported $\gamma\text{-}Fe_2O_3$ nanoparticles modified with

lipase.[24] This allows to add fresh solvent including cholesterol substrate in order to start again the enzymatic process in a new cycle, using a magnet to separate the supported nanoparticles from solution between consecutive cycles. Interestingly, the efficiency of the sepiolite-magnetite NPs/PC-COx bioreactor is well preserved, remaining close to 90% after more than 10 consecutive cycles, as shown in figure S4. This new type of superparamagnetic bioreactor allows a very useful and fast non-degrading recovery step in comparison to conventional centrifugation based processes. In conclusion, the procedure here reported consists in the use of a porous solid as support of a non-aqueous ferrofluid, in order to provide the solid with superparamagnetic behaviour while maintaining its adsorption ability. These new systems open up ways for a wide range of multifunctional materials that can be built from these superparamagnetic porous platforms. The reported examples, i.e. magnetic hybrid buckypapers and bioreactors, are based on sepiolite-NPs but the approach can be enlarged to other modified porous solids such as active carbons, silica and zeolites of wide incidence in different applications

*Experimental*

Materials and methods: details on reagents and other chemicals used in the preparation of the materials, as well as information of experimental techniques for characterization are given in the Supplementary Information (SI).

Samples preparation:

Magnetite NPs and ferrofluid preparations were prepared following an approach based on that previously described.[2] The ferrofluid was prepared using n-heptane and further assembled to the porous materials as reported in ref. 1. In general, materials were

prepared with a relative content of magnetite-NPs/porous solid 1:1 (w/w) from the ferrofluid and the solvent removed by evaporation.

Buckypapers preparation: 100 mg of sepiolite modified with magnetite NPs (1:1) was used to assemble to 5 mg of MWCNTs in 20 ml of double distilled water under ultrasonic irradiation (VC750 Sonics Vibra-Cell, 20kHz), then filtered under vacuum obtaining the self-supported samples in the form of black thin disks.

Bioreactor preparation: Magnetic COx-bioreactors were prepared from sepiolite containing 35% $Fe_3O_4$/oleic acid NPs as solid support. Lipid modification was achieved by immersing 50 mg of this sample in 25ml of a 5mM PC solution in ethanol. The solid was separated by means of a magnet, washed twice in bidistilled water and stored as 10mg/ml suspension in 50mM phosphate buffer (PB) of pH 7.0 until usage. COx was immobilized from a 2000 µg/ml stock solution by contacting 2mg support with 160 µg COx. After 2h incubation at ambient temperature the solid was separated and washed with copious amounts of PB. Details on enzymatic activity assay are given in SI.

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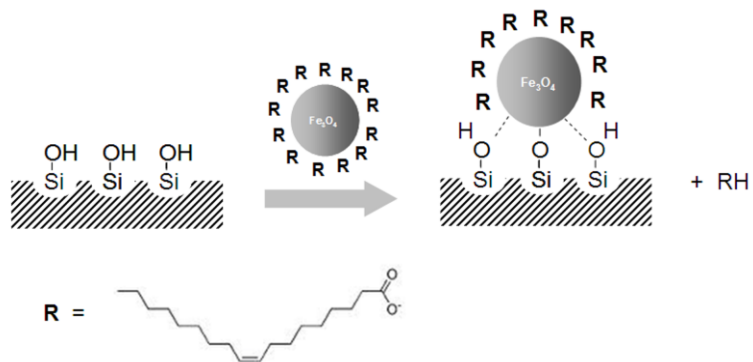
**Scheme 1**. Proposed mechanism for NPs immobilization through interaction**s** with surface hydroxyl groups, in the example silanol groups on sepiolite surface.
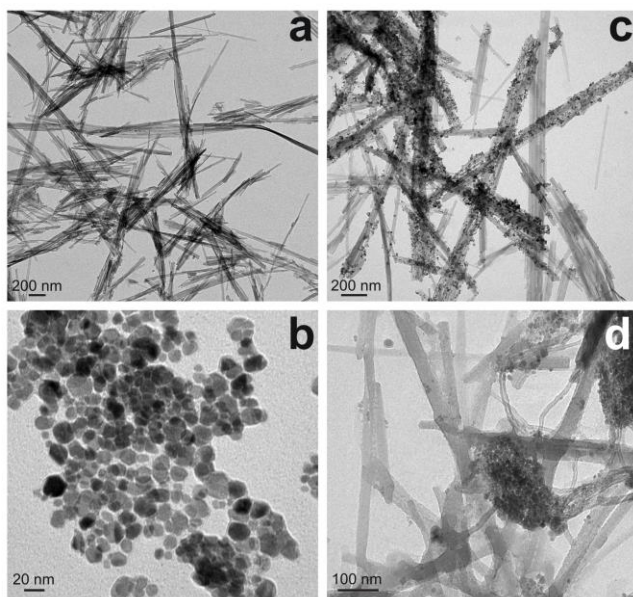


**Figure 1**. TEM images of (a) pristine sepiolite, (b) oleic acid-magnetite NPs, (c) oleic acid-magnetite NPs supported on sepiolite (50% w/w) and (d) hybrid buckypaper based on sepiolite-NPs (50% w/w) assembled to MWCNTs (20:1 ratio)
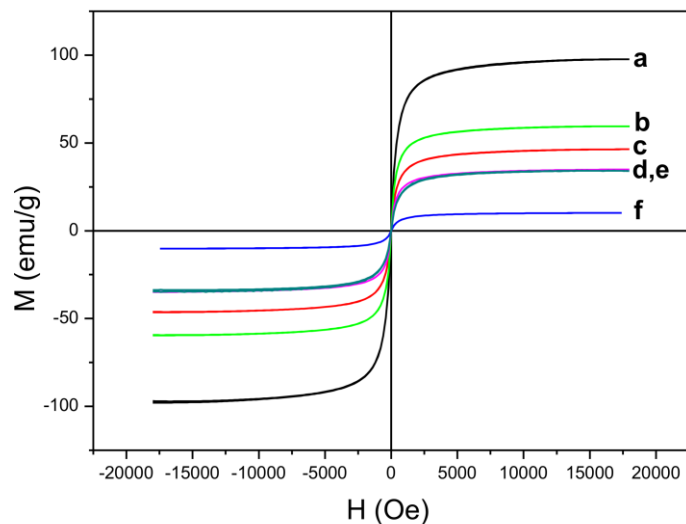
**Figure 2** Magnetization curves at room temperature of (a) oleic acid-magnetite NPs, (b) sepiolite after assembling oleic acid-magnetite NPs in 50% and (c) 35% weight content, (d) sample b assembled to MWCNTs in 20:1 ratio, (e) zeolite ZSM5 after assembly oleic acid-magnetite NPs in 50% weight content and (f) sample c incorporating a PC lipid monolayer.
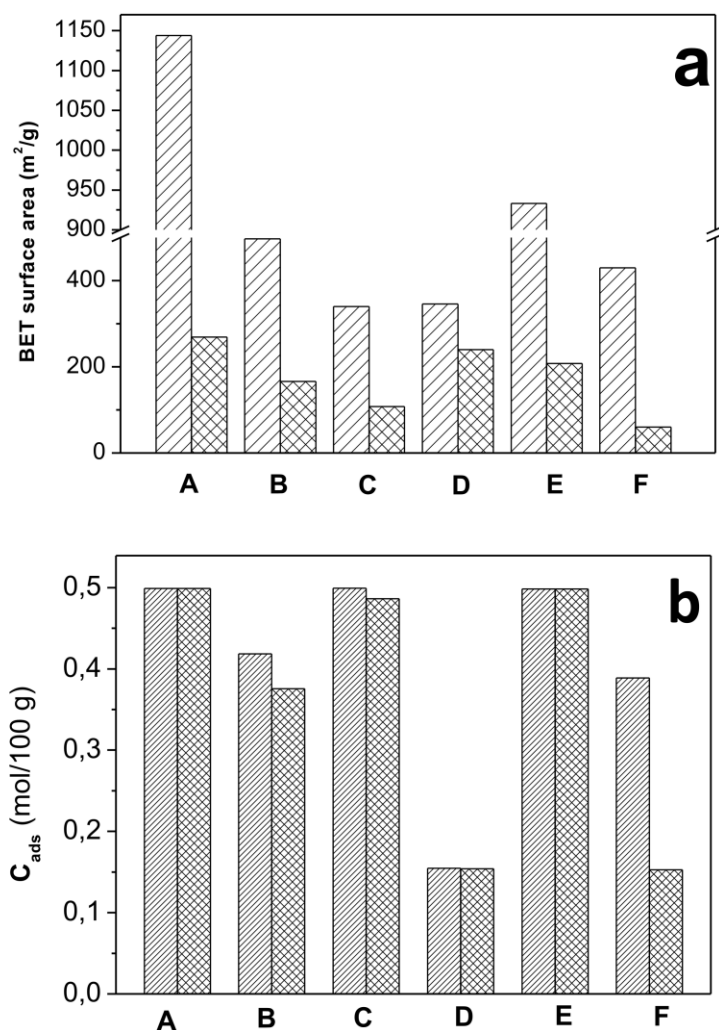
**Figure 3.** (a) BET specific surface area of (A) Norit® RO 0.8 active carbon pellets, (B) Merck60 silica gel, (C) sepiolite, (D) LA3P silica-alumina, (E) Fluesorb B active carbon powder, and (F) ZSM5 zeolite, before (left) and after (right) assembly to oleic acid-magnetite NPs in a 1:1 ratio; (b) adsorption of MB dye for the same samples.

**Multifunctional superparamagnetic materials** based on porous solids have been prepared by an innovative, simple and easy procedure that implies their treatment with ferrofluids. Results support that this approach can be especially useful for technological, environmental and biomedical applications for replacing separation processes by conventional filtration or centrifugation. This methodology has been applied for the preparation of *hybrid* buckypapers, showing that it can be also relevant for the development of other superparamagnetic multifunctional materials for diverse advanced applications.

Keywords: Hybrid materials, Superparamagnetic solids, Adsorbents, Porous Solids, Clay materials, Sepiolite, Zeolites, Carbonaceous materials, CNTs, Bioreactors

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**Multifunctional porous materials through ferrofluids**
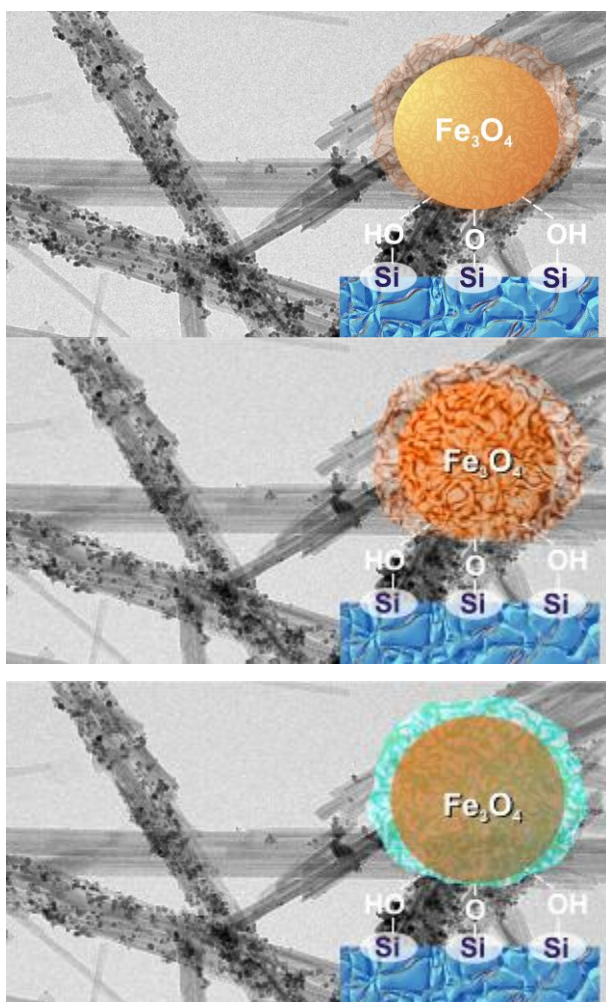
**Supporting information**

**Supporting information materials and methods:** Oleic acid (99% purity),

$FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ (Sigma-Aldrich), ultra pure water (resistivity of 18.2

$M\Omega \cdot cm$) and ammonia aqueous solution (28% purity, Fluka) were used in the synthesis

of the magnetite NPs. The solid supports were: sepiolite of rheological grade (Pangel® ,

Tolsa SA), Merck 60 silica gel (Merck), ZSM5 zeolite (gift by Prof. G. Poncelet, Univ.

Louvain), LA-3P silica-alumina catalyst (Ketjen) and active carbons commercialised as

Norit® RO 0.8 pellets (Sigma-Aldrich) and Fluesorb B powder (Chemviron Carbon).

CVD grown MWCNTs (> 95% carbon content, 10 nm average diameter and 1.5 μm

average length, from Dropsens SA). Cholesterol oxidase (COx) from *Brevibacterium*

*sp.* (33 U/mg solid), cholesterol (99%), and Triton® X-100 (Sigma-Aldrich). L-α-

phosphatidylcholine (PC) from soy bean, commercialized as Emulmetik$^{TM}$ 930, was

kindly provided as a gift from Lucas Meyer Cosmetics, Germany, and used as received.

Other reagents: 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS),

peroxidase from horseradish (HRP) and $KH_2PO_4$ (SigmaAldrich). All the materials

were used as received, i.e. without further treatments.

Resulting materials were characterized by means of: XRD (Bruker D8 equiped with a

SOLX detector), FTIR spectroscopy (BRUKER IFS 66v/S); CHNS elemental chemical

microanalysis (PerkinElmer 2400 series II analyser); TG-DTA thermal analysis (SEIKO

SSC/5200) under air and $N_2$ dynamic atmospheres; TEM (LEO-910 microscope

operating at 80 kV and JEOL 2000 FXII that works at 200 kV)) and FE-SEM (FEI-

NOVA NANOSEM 230) microscopies. Textural properties were analyzed by $N_2$

adsorption/desorption experiments recording complete isotherms (Micromeritics ASAP

2010 analyzer) and in some cases, "one-point BET" specific surface areas were

determined from adsorption analysis at 77K (Micromeritics Flowsorb II 2300). UV-vis

spectrophotometric determinations for evaluation of MB adsorption and enzyme activity were carried out in an UV-vis Shimadzu, UV1201 spectrometer applying the Lambert-Beer law for determination of concentrations. VSM measurements were carried out at room temperature applying a variable magnetic field from –18000 to 18000 Oe in a vibrating sample magnetometer with an ADE magnetic system (model EV7). Conductivity measurements were performed in DC mode using an in-line four probe setup.

**Supporting information activity assay:** Enzymatic activity of COx-bioreactors was determined from spectrophotometric experiments. First, cholesterol stock solutions were prepared as 0.1M in iso-propanol. Working solutions of 0.5mM were prepared in PB containing 1%wt. Triton X-100 by adding an aliquot of cholesterol stock solution under vigorous stirring. The mixture was warmed up slowly to 60ºC until the solution became clear. The bioreactor (2mg) was suspended in 2ml PB and an aliquot of cholesterol working solution was added. The mixture was thermostated for 7min at 25ºC in a water bath shaker. The solid was then separated using a magnet and the supernatant was analyzed for the amount of enzymatically produced $H_2O_2$ using a Shimadzu UV-2401 PC UV-Vis spectrophotometer. Therefore, 1ml of supernatant was mixed with 10µl of 10 mM ABTS and 50 µl of 23 µM HRP and brought up to 2ml with PB. After 2min. a UV-Vis spectrum was collected and the absorbance at 728nm was measured. The recyclability of bioreactors was evaluated. After each addition of 31µM cholesterol, the activity assay was conducted as described above. The bioreactor was separated by means of a magnet and washed thoroughly in PB before starting a new catalysis cycle.
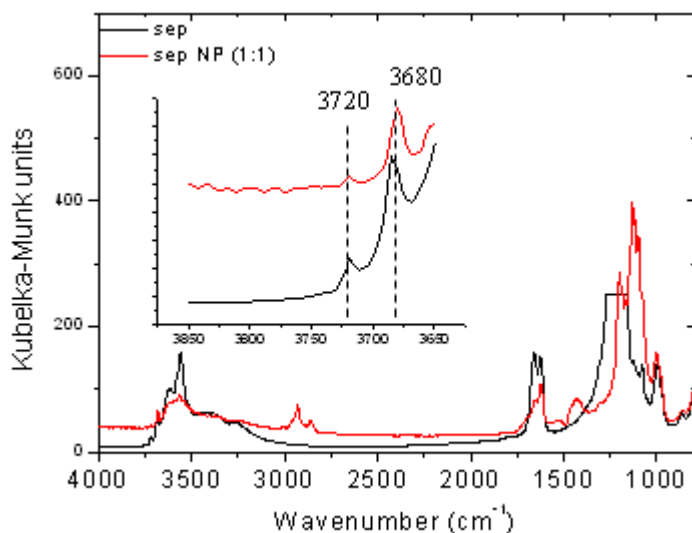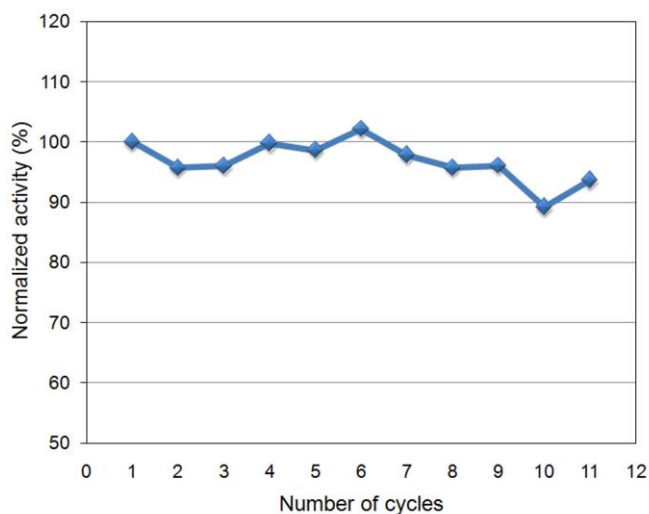
**Supporting information figure S1**. FTIR spectra of sepiolite (sep) and sepiolite/ oleic acid-magnetite NPs in a 1:1 (w/w) ratio (sep NP (1:1)), recorded using reflectance diffuse mode. The inset shows the strong decrease in intensity of the $\nu_{O-H}$ vibration band ascribed to Si-OH groups in the surface of sepiolite upon assembly of the oleic acid-magnetite NPs
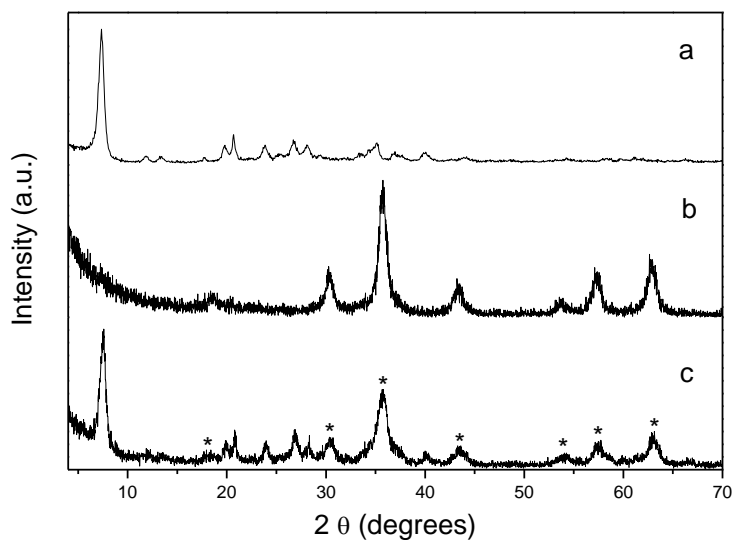


**Supporting information figure S2**. XRD patterns of (a) sepiolite, (b) oleic acid-magnetite NPs and (c) sepiolite-oleic acid NPs in a 1:1 (w/w) ratio. Peaks marked with * in pattern **c** are ascribed to the assembled magnetite NPs.
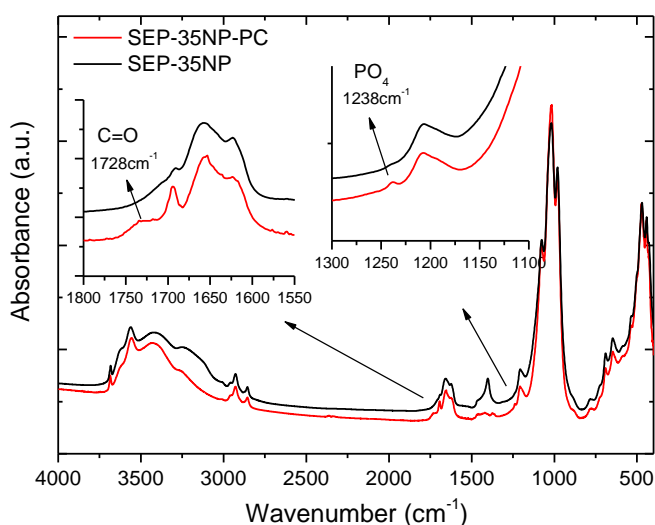
**Supporting information figure S3.** FTIR spectra of sepiolite assembled to oleic acid-magnetite NPs in 35% weight content, before (SEP-35NP) and after modification with PC phospholipids (SEP-35NP-PC), prepared in KBr pellets and recorded in transmission mode. The insets show the phospholipid specific bands of $PO_4$ and C=O.



**Supporting information figure S4.** Reusability of a magnetic bioreactor for cholesterol transformation based on sepiolite assembly oleic acid-magnetite NPs in 35% weight content incorporating PC phospholipids (SEP-35NP-PC material)

Each cycle comprises the following steps: i) addition of cholesterol substrate and catalytic reaction, ii) separation of magnetic catalyst by using a magnet and full extraction of the reaction media, ii) quantification of catalytic reaction products by UV-Vis using ABTS and HRP, iii) washing step of the catalyst with PBS, iv) addition of fresh solvent including cholesterol substrate and proceeding with step i)

The separation of the catalyst was carried out not by extraction of the solids themselves by means of a magnet but by applying the magnet to the external surface of the vial in order to retain the particles fix in the vial. In this way, the reaction media can be

conveniently extracted by a pipette and the remaining particles can be easily re-suspended in fresh solution.

**Supporting information video S1.** Adsorption of MB in a 5 x 10-4 M solution by sepiolite- oleic acid NPs in a 1:1 (w/w) ratio.