

1 **Title:** Synthesis and characterization of magnetic chitosan microspheres as low-density
2 and low-biototoxicity adsorbents for lake restoration

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16 **Abstract**

17 We propose a novel magnetic adsorbent for optimal Phosphorus (P) removal from the
18 upper sediment layers. For this aim, magnetic chitosan microparticles were prepared
19 using a reverse-phase suspension cross-linking technique. The resulting particles and
20 suspensions were characterized using scanning electron microscopy, X-ray powder dif-
21 fraction, Fourier transform infrared spectroscopy, magnetometry, thermogravimetric
22 analysis, electrophoretic mobility and turbidity measurements. The hybrids are multi-
23 core particles consisting of well dispersed magnetite nanoparticles (approx. 10 % w/w)
24 homogeneously distributed within the biopolymer matrix. These microparticles can be
25 easily separated from the water column and sediment using magnetic field gradients.
26 Their P adsorption capacity is evaluated in batch conditions resulting in a maximum P
27 adsorption capacity of $M_L = 4.84 \text{ mg g}^{-1}$ at pH = 7. We demonstrate that these particles
28 are excellent candidates to remove P from water column and also P mobile from the
29 upper sediment layers due to two main reasons: they sediment slower and present lower
30 potential toxicity (due to a their larger size) than conventional iron/iron oxide micropar-
31 ticles previously proposed for lake restoration.

32 **1. Introduction**

33 Enrichment of phosphorus (P) is considered the main cause of eutrophication of inland
34 waters (Carpenter, 2005). In situations in which P release from sediment (internal P
35 load) impedes lake recovery once external P load has been reduced, in-lake addition of
36 P-binding adsorbents appears as an advisable alternative for lake management (Cooke et
37 al., 2005; Søndergaard et al., 2003). Z2G1 or Aqual-P®, Phoslock®, calcium, iron (Fe)
38 or alum salts have been demonstrated to increase sediment P-sorption capacity (Cooke
39 et al., 2005; Dittrich et al., 2011; Gibbs et al., 2011; Robb et al., 2003; Søndergaard et
40 al., 2003). Although some of these techniques could represent a long-lasting sink for P,
41 changes in physicochemical conditions or resuspension events can lead to undesirable P
42 release to water column along with toxic substances stemming from the adsorbents
43 composition (Egemose et al., 2009; Zamparas and Zacharias, 2014).

44 In the last decades, magnetic nano- and microparticles have attracted special attention to
45 adsorb contaminants from waters and soils due to their easy separation and recovery
46 from the medium for further reuse. This obviously leads to cost savings and to the re-
47 duction of the contact time with biota (Crane and Scott, 2012; Gómez-Pastora et al.,
48 2014; Huber, 2005; Lu et al., 2007; Tang and Lo, 2013). With this in mind, more re-
49 cently, these magnetic particles have been successfully used for P removal in the con-
50 text of lake restoration and improvement of water quality (Choi et al., 2016; Daou et al.,
51 2007; de Bashan and Bashan, 2004; de Vicente et al., 2010; Funes et al., 2016, 2014;
52 Lai et al., 2016; Long et al., 2011; Shaikh and Dixit, 1992).

53 Apart from high P adsorption capacity and reusability, magnetic particles must meet
54 other stringent requirements for lake restoration applications. Particle size has very im-
55 portant implications not only in adsorption capacity but also in biological toxicity.

56 Small (nano) particles are preferred to enhance adsorption capacity (because of their
57 large surface area) (Yavuz et al., 2006). However, there are evidences of toxicological
58 effects on aquatic organisms (Oberdörster, 2004); because cladocerans such as *Daphnia*
59 *sp.* filter large volumes of water, considerable amounts of nanoparticles can be ingested
60 by them, having negative consequences in their physiology and also in upper levels of
61 the food chain (Lovern and Klaper, 2006, Zhu et al., 2010). Unfortunately, nanoparticles
62 can also negatively affect aquatic biota by adsorption on their carapaces and filtrations
63 apparatus (Baumann et al., 2014). Finally, Fe dissolution is another indirect effect of
64 using Fe based nanoparticles which can led to phytotoxicity (Keller et al., 2012).

65 Of outstanding importance in this work is particle density. Actually, particle density is a
66 key factor driving adsorbent distribution through the sediment profile and determines its
67 availability for P adsorption. It is worth to stress here that sedimentary P mobile, which
68 is the target P pool, mostly concentrates within the first 10 cm of sediment and de-
69 creases rapidly with depth (Reitzel et al., 2005). From this perspective, very large den-
70 sity particles are not desirable because they sink deeper in sediment being not available
71 for P adsorption. Moreover, sinking of magnetic particles into deeper layers may con-
72 tribute to intense sediment resuspension when removing the magnetic particles with the
73 magnet causing undesirable effects (Søndergaard et al., 1992). In addition, large density
74 particles may sink into the deepest sediment layers where magnetophoretic forces ex-
75 erted by the magnets could not be large enough to overcome opposing surface (cohe-
76 sive) forces within the sediment making the recovery process inefficient (Yavuz et al.,
77 2006). Thus, a balance between high P removal efficiency (high affinity and large sur-
78 face area), low bio-toxicity (biocompatible components and large particle size to diffi-
79 cult the ingestion by biota) and low density (to remain in the first 10 cm of sediment)
80 must be pursued (Philippova et al., 2011; Reddy and Lee, 2013).

81 In this work, we fabricate hybrid magnetic particles (magnetic chitosan microspheres,
82 MCMs) that accomplish the three requirements discussed in paragraph above. We fol-
83 low a novel approach that consists in the encapsulation of magnetic multicores within a
84 biopolymer spherical matrix that prevents Fe dissolution, is non-toxic and less dense
85 (than traditional magnetic adsorbents), but still exhibiting a large adsorption capacity
86 for lake restoration applications. Furthermore, by using a chitosan matrix we introduce
87 versatile adsorption properties (apart from the traditional specific adsorption mechanism
88 between P and Fe oxides) that can be controlled through the cross-linker concentration
89 due to the presence of amine and hydroxyl groups and therefore pH dependent electro-
90 static interactions.

91 **2. Materials and Methods**

92 All reagents were of analytical grade and used without further purification. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$,
93 ethylene glycol (EG) and sodium acetate (NaAc) were supplied from VWR (AnalaR-
94 Normapur). Trisodium citrate (Na_3Cit) was obtained from Carlo Erba. Chitosan (low
95 molecular weight; 75-80 % deacetylated), glutaraldehyde (8%), mineral oil, Span 80
96 and petroleum ether were acquired from Sigma Aldrich. Acetic acid (5%) and acetone
97 were obtained from Panreac. Finally, carbonyl Fe microparticles were obtained from
98 BASF (grade HQ, average diameter = 800 nm, Germany).

99 *2.1 Synthesis of magnetite (Fe_3O_4) nanoparticles*

100 There are several routes for the synthesis of Fe_3O_4 depending on the particle size to be
101 obtained. For instance, Fe_3O_4 nanoparticles of typical sizes in the range ~ 10 nm can be
102 prepared by the Massart's method (Massart, 1981). In this case, Fe^{+2} and Fe^{+3} are
103 coprecipitated in an alkaline solution. Another option to obtain Fe_3O_4 particles in the

104 micronsized range, ~ 1000 nm, is to follow Sugimoto and Matijevic (1980). In this case,
105 Fe^{+2} oxidation is slowed down by a nitrate salt allowing the particles to grow larger. In
106 this particular work, we are interested in particles having an intermediate particle size (\sim
107 100 nm): large enough to respond to magnetophoretic forces and small enough for a
108 large surface activity and hence adsorption capacity.

109 We used a solvothermal method following the recommendations by Liu et al. (2009a)
110 with slight modifications. Briefly, 3.38 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.25 mol L^{-1}) and 0.5 g of
111 Na_3Cit (34 mmol L^{-1}) were dissolved in 50 mL of ethylene glycol by using a centrifugal
112 mixer (3 min at 1000 rpm). NaAc (3 g) was then added and the suspension was mixed
113 again (15 min at 1000 rpm). Next, the mixture was sealed in a Teflon-lined stainless-
114 steel autoclave (125 mL capacity) and heated at $215 \text{ }^\circ\text{C}$ for 10 h. The obtained magnetic
115 nanoparticles were washed several times with ethanol and dried at $50 \text{ }^\circ\text{C}$ for 4 hours.

116 *2.2 Preparation of magnetic chitosan microspheres (MCMs)*

117 MCMs were fabricated by a reverse-phase suspension cross-linking technique follow-
118 ing Jiang et al. (2005) with slight modifications. First, previously synthesized Fe_3O_4
119 powder (0.082 g) was dispersed via ultrasonication in a solution of chitosan (0.25 g) in
120 5% acetic acid (10 mL) for 10 min. The Fe_3O_4 :chitosan ratio (by weight) was 1:3. Next,
121 10 mL of this suspension were added drop-wise to a mixture of mineral oil (37.5 mL)
122 and Span 80 (2.5 g) as a surfactant. During all the process, the formed emulsion was
123 stirred with a mechanical stirrer at 2500 rpm for 30 min. Then, 5 mL of glutaraldehyde
124 were added dropwise and the dispersion was stirred for another 1 h at $40 \text{ }^\circ\text{C}$ to promote
125 the cross-linking. Finally, the magnetic microspheres were separated from the oil phase
126 by applying a magnet for 30 min and washed several times with petroleum ether and
127 acetone. MCMs were then dried at $50 \text{ }^\circ\text{C}$ for 4 h and stored until use. Chitosan micro-

128 spheres (CMs) were prepared following the same procedure as MCMs without addition
129 of Fe₃O₄. CMs were separated from oil phase by centrifugation at 3000 rpm for 5 min,
130 washed and stored as in MCMs procedure.

131 *2.3 Characterization of the hybrid particles*

132 Size and surface morphology of Fe₃O₄, MCMs and CMs particles were elucidated with
133 high-resolution field-emission scanning electron microscopy (FIB-FESEM, AURIGA,
134 Carl Zeiss SMT Inc.). Environmental scanning electron microscopy (ESEM, FEI
135 Quanta 400) was used to determine internal distribution of Fe₃O₄ particles within
136 MCMs. Samples were embedded within an Epofix resin and cross-sectioned. Crystal
137 structure of Fe₃O₄ and MCMs was analyzed with a Bruker D8 Advance X-Ray diffrac-
138 tometer (XRD) with scattering angle (2θ) of 0-90° using Cu-Kα as incident radiation (λ
139 = 0.1540 Å) at 40 kV. Fourier transform infrared spectroscopy (FT-IR) was recorded
140 between 4000-400 cm⁻¹ on a JASCO 6200 spectrophotometer in attenuated total reflec-
141 tion (ATR) mode to observe interactions between functional groups of the different syn-
142 thesized systems. Samples were prepared with KBr disks. Thermal stability of the dif-
143 ferent composites was analyzed by a thermogravimetric analyzer (METTLER TOLEDO
144 mod.DCS1). Samples were heated from 37 to 950 °C with a heating rate of 10 °C min⁻¹
145 in N₂ atmosphere. The magnetization measurements of the composites were performed
146 with a SQUID magnetometer (MPMS XL, Quantum Desing) at room temperature. The
147 external field was swept from -4000 to 4000 kA m⁻¹. Electrophoretic mobility of the
148 composites was determined using a Zetasizer Nano Z (Malvern instruments, UK) at 25
149 °C. To minimize sedimentation of magnetic particles under gravity, prior to the test, the
150 samples were sonicated for 5 min. Samples for measuring electrophoretic mobility were
151 prepared as follows: a suspension of Fe₃O₄ (0.1 g L⁻¹) or MCMs (0.72 gL⁻¹) or CMs

152 (0.62 g L⁻¹) with 3 mM NaHCO₃ was agitated for 24 h in a horizontal shaker (150 rpm)
153 at different pH values. Afterwards, pH was readjusted and the suspensions were made
154 up to 25 mL of volume. Turbidity measurements were performed using a Turbiscan MA
155 2000 (Formulation, France) in order to compare sedimentation rates of MCMs and
156 carbonyl Fe microparticles used by de Vicente et al. (2010). Briefly, dispersions were
157 placed in a cylindrical plastic cell which was scanned from the bottom to the top with an
158 incident near infrared light source ($\lambda_{\text{air}} = 850$ nm; Wulff-Pérez et al., 2009). Measure-
159 ments reported here only consider transmission data -as a function of sample height and
160 time-. Samples were prepared by mixing a suspension of carbonyl Fe microparticles
161 (0.1 g L⁻¹) or MCMs (0.72 g L⁻¹) with 3 mM NaHCO₃ under agitation for 24 h at pH 7
162 in a final volume of 25 mL.

163 *2.4 Batch adsorption experiments*

164 Fe₃O₄, MCMs and CMs stock suspensions were prepared by mixing either 100 mg or
165 720 mg or 620 mg, respectively, with 100 mL of distilled water in a polyethylene con-
166 tainer. Suspensions were sonicated for 5 min prior to their use for adsorption experi-
167 ments to ensure homogeneity of the dispersion. As 2.5 mL of the stock solution (1 g
168 Fe₃O₄ L⁻¹; 7.2 g MCMs L⁻¹; 6.2 g CMs L⁻¹) were added to a final volume of 25 mL, the
169 final concentration for each suspension was 0.1 g Fe₃O₄ L⁻¹, 0.72 g MCMs L⁻¹ and 0.62
170 g CMs L⁻¹.

171 To investigate the effect of pH on P removal efficiency by MCMs, 2.5 mL of a MCMs
172 stock suspension (7.2 g L⁻¹) were mixed with 20 mL of 3 mM NaHCO₃ (acting as a
173 buffer) and shaken for 24 h in a horizontal shaker (150 rpm). Then, 2 mL of a 0.125
174 mM P stock solution were added to each tube and pH was adjusted to 7, 8 and 9. Sus-
175 pensions were shaken (150 rpm) for 24 h. After this time, pH was readjusted and the

176 volume was made up to 25 mL with 3 mM NaHCO₃. Initial P concentration accounted
177 for 0.01 mM P. A magnetic separation gradient (PASCO scientific; EM-8641) was ap-
178 plied for 5 min and the supernatant was filtered to measure P by spectrophotometric
179 procedure according to Murphy and Riley (1962).

180 Maximum P adsorption capacity was tested by adding 2.5 mL of the corresponding
181 stock suspension to 20 mL 3 mM NaHCO₃ in 25 mL centrifuge tubes. Final adsorbent
182 concentrations of Fe₃O₄ and CMs isotherms were fixed according to Fe₃O₄ and chitosan
183 content in MCMs as obtained from magnetometry tests. Suspensions were shaken (150
184 rpm) for 24 h and then, 2 mL of KH₂PO₄ solutions with P concentrations ranging from
185 0.125 to 2 mM P were added. The pH value was adjusted to 7 and suspensions were
186 shaken again (150 rpm) for 24 h. Afterwards, pH was readjusted and the volume was
187 made up to 25 mL with 3 mM NaHCO₃. The magnetic separation gradient was applied
188 for 5 min and P determination was carried out as previously mentioned. The equilibrium
189 adsorption capacity of P, q , was calculated as follows:

$$190 \quad q = \frac{C_0 - C_e}{M_a} V \quad [1]$$

191 where C_0 and C_e are the initial and equilibrium P concentration (mg L⁻¹), respectively,
192 M_a is the mass of adsorbent in grams and V is the total volume of the suspension (L).

193 **3. Results and discussion**

194 *3.1 Characterization of composites*

195 Fig. S1 (supplemental material) shows a typical SEM image of the Fe₃O₄ particles. As
196 observed, they are quasi-spherical in shape and uniform in size with a mean diameter of
197 about 280 nm. A detailed description of their morphological characteristics is provided

198 in Table S1 (supplemental material). Higher magnification images indicate that Fe_3O_4
199 consisted of clusters of small nanocrystals (Fig. S1 inset). CMs were also visualized
200 under the microscope. As demonstrated in Fig. S2 (supplemental material), they were
201 spherical in shape but significantly larger, and more polydisperse, than Fe_3O_4 nanoparti-
202 cles (~3200 nm average number diameter with a polydispersity index of 1.7, Table S1).
203 Fig. 1a reveals that MCMs were also polydisperse and spherical in shape with larger
204 diameter than CMs (average number diameter ~4800 nm). More details on the morpho-
205 logical characteristics of the particles can be found in Table S1. In contrast to CMs,
206 MCMs showed a grainy surface (Fig. 1a inset) whereas CMs surface was smooth (Fig.
207 S2 inset). The cross-sectioned MCMs depicted in Fig. 1b revealed a homogeneous dis-
208 tribution of Fe_3O_4 nanoparticles within the chitosan microspheres indicating that the
209 embedding process resulted in little Fe_3O_4 aggregation.

210 Fig. S3 (supplemental material) shows the XRD patterns of Fe_3O_4 and MCMs particles.
211 Six diffraction peaks at $2\theta = 30.18^\circ$, 35.5° , 43.16° , 53.37° , 56.86° and 62.71° were
212 clearly identified in both composites. These peaks fit to Fe_3O_4 according to the Joint
213 Committee on Powder Diffraction Standards (JPCS) database but it is difficult to differ-
214 entiate between Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ since both have similar spinel structures and there-
215 fore similar XRD patterns (Zhou et al., 2002). The broad peak at $2\theta = 20.53^\circ$ is ascribed
216 to the presence of chitosan (Kyzas and Deliyanni, 2013). Results corroborate that Fe_3O_4
217 is embedded within the chitosan matrix and the lower intensity found in MCMs com-
218 pared to bare Fe_3O_4 comes from the amorphous chitosan structure.

219 FT-IR spectra of the synthesized Fe_3O_4 , MCMs and CMs particles are shown in Fig. S4
220 (supplemental material). For comparison, this figure also includes the spectra corre-
221 sponding to chitosan powder. The sharp peak at 587 cm^{-1} is assigned to vibrations of

222 Fe-O bonds in Fe₃O₄ (Tian et al., 2011). This characteristic peak is also present in
223 MCMs but shifts to 621 cm⁻¹. The absorptions at 1607 and 1385 cm⁻¹ in Fe₃O₄ are at-
224 tributed to stretching vibrations of C=O bonds indicating the presence of carboxyl
225 groups coming from a thin layer of Na₃Cit stabilizer on the surface of Fe₃O₄ particles in
226 agreement with Liu et al. (2009a) . For chitosan powder the N-H band vibration is found
227 at around 1639 cm⁻¹ (Agnihotri and Aminabhavi, 2004). In MCMs and CMs a new peak
228 appears at around 1563 cm⁻¹ which is ascribed to ethylenic bond (C=N) indicating the
229 reaction of carboxyl groups of glutaraldehyde and amine groups of chitosan to form the
230 *Schiff base* (Kumar et al., 2009; Li et al., 2013; Monteiro and Airoidi, 1999). The ab-
231 sorption band at 1720 cm⁻¹ indicates the presence of free aldehydic group in CMs
232 whereas no evidence was found for MCMs (Monier et al., 2012) . A peak around 2923
233 cm⁻¹ is related to stretching vibration of C-H bonds (Chen and Park, 2003; Zhang et al.,
234 2011). The broad peak at 3437 cm⁻¹ assigned to O-H bond stretching or N-H bond
235 stretching increased in intensity in the case of MCMs possibly indicating high propor-
236 tion of unreacted free amine and/or hydroxyl groups compared to CMs (Iyengar et al.,
237 2014; Monier et al., 2012).

238 Magnetization curves of the Fe₃O₄ and MCMs particles at 293 K are represented in Fig.
239 2. Fe₃O₄ and MCMs behave as soft magnetic materials with no coercivity and rema-
240 nence pointing out that the single-domain magnetic nanoparticles persisted in MCMs
241 (Zhang et al., 2010). The saturation magnetization (*M_s*) of the synthesized Fe₃O₄ (64
242 emu g⁻¹) is well below the theoretical value for bulk Fe₃O₄ (84 emu g⁻¹) (Philippova et
243 al., 2011) but close to that obtained by Liu et al. (2009a) (73 emu g⁻¹). This is explained
244 by a reduction in crystallinity due to the presence of Na₃Cit during synthesis process
245 and surface organic coating (Liu et al., 2009a). Obviously, embedding magnetic parti-
246 cles within the chitosan matrix results in reduction of *M_s* value (Gómez-Pastora et al.,

247 2014; Liu et al., 2009b). Using the reference value for the synthesized Fe_3O_4 , the mag-
248 netic content of the hybrid particles is approx. 14% w/w. This magnetite loading is
249 clearly lower than 33 % (i.e. Fe_3O_4 :chitosan ratio of 1:3) hence suggesting that some
250 magnetic nanoparticles are not encapsulated within the chitosan microspheres during the
251 emulsification process. As will be seen later, MCMs retained a magnetic character that
252 is enough to ensure separation from the medium when applying an external magnetic
253 field (see below).

254 TGA measurements for the different particles are summarized in Fig. S5 (supplemental
255 material). Fe_3O_4 showed an important weight loss (16 %) in the range of 37-678 °C
256 which is slightly higher than that reported by Liu et al. (2009a). A weight loss of about
257 3% occurred from 37 °C to 180 °C. This corresponds to the evaporation of water mole-
258 cules adsorbed on the surface of Fe_3O_4 (Cai and Wan, 2007) . The most significant
259 weight loss (13%) occurred in the interval 180-678 °C. This is attributed to the removal
260 of the Na_3Cit organic compound used during Fe_3O_4 synthesis (Liu et al., 2009a). Fi-
261 nally, a slight weight gain of approximately 2 % was observed in the range 678-950 °C,
262 attributed to the oxidation of Fe_3O_4 to $\gamma\text{-Fe}_2\text{O}_3$ (Tian et al., 2011) . MCMs showed a
263 11% of weight loss below 180 °C due to the removal of adsorbed water. However, the
264 most significant degradation was at 180-550 °C corresponding to breakage of chitosan
265 chains (Kalkan et al., 2012) and this continues until 950 °C. CMs started to degrade at
266 lower temperature than pure chitosan indicating that cross-linking reduces thermal sta-
267 bility (Li et al., 2013; Neto et al., 2005). Also, CMs showed less thermal stability than
268 MCMs due to the absence of Fe_3O_4 . Chitosan powder showed an overall 76% of weight
269 loss. A slight weight loss of 9 % is observed below 100 °C and ascribed to the release of
270 adsorbed water molecules (Kumar and Koh, 2012). However, it is mostly degraded be-
271 tween 300-500 °C (Kalkan et al., 2012). The magnetic content of the hybrid particles as

272 estimated from TGA measurements is approx. 9 % w/w. This result is in reasonably
273 good agreement with magnetometry measurements reported in the paragraph above.

274 3.2 Turbidity measurements: sedimentation

275 The penetration of the particles within the sediment layer depends on the sedimentation
276 rate. Generally speaking, the sedimentation rate V of a colloidal suspension is governed
277 by the density mismatch between the particles ρ_p and the carrier fluid ρ_c , particle di-
278 ameter d , gravity constant g , continuous phase dynamic viscosity ν and particle volume
279 fraction ϕ (Mills and Snabre, 1994) :

$$280 \quad V = \frac{|\rho_p - \rho_c| g d^2}{18 \nu \rho_c} \frac{1 - \phi}{1 + \frac{4.6 \phi}{1 - \phi^3}} \quad [2]$$

281 In the case of MCMs particles, the $|\rho_p - \rho_c|$ term clearly approaches to zero (if com-
282 pared to classical Fe particles) and hence the sedimentation rate is expected to be re-
283 duced if the other parameters remain fixed in Equation [2]. However, the diameter of
284 the MCMs particles is approximately 5 times larger than carbonyl Fe (see Table S1) and
285 this contributes, as well, to increase the sedimentation rate.

286 As a result, turbidity measurements were also performed to interrogate the sedimenta-
287 tion characteristics of MCMs suspensions and ascertain whether they sediment slower
288 than carbonyl Fe. For completeness carbonyl Fe suspensions were also measured at the
289 same particle concentration (0.72 g L^{-1}). A sedimentation process was clearly observed
290 in both systems, demonstrated by an increase in the transmission profiles (clarification)
291 at the top of the sample with a concomitant decrease of transmission at the bottom of
292 the sample (Fig. S6a and S6b, supplemental material). Clarification in the whole height
293 of the tube also evidenced aggregation of the dispersed particles in both suspensions,

294 although it was more intense for carbonyl Fe microparticles. Fig. 3 represents the time
295 evolution of the sedimentation front (H/H_0) for the two suspensions. In the case of car-
296 bonyl Fe particles, the sedimentation front experienced a marked decrease over time
297 reaching a constant value of 20% after 5 min whereas MCMs showed a much lower
298 sedimentation over time reaching the constant value of 20% after 30 min.

299 These results demonstrate that the sedimentation rate of MCMs is slower than carbonyl
300 Fe microparticles despite of their higher particle diameter (average diameter: carbonyl
301 Fe microparticles = 800 nm; MCMs = 4800 nm). This is explained by the lower density
302 of MCMs compared to carbonyl Fe microparticles.

303 *3.3 P removal efficiency by MCMs: effect of pH*

304 In general, pH has an important influence on the nature of the adsorbent and adsorbate
305 (Gómez-Pastora et al., 2014) . Therefore, the P removal efficiency by MCMs is ex-
306 pected to be influenced by the pH. Results obtained for MCMs are summarized in Fig.
307 4. This figure shows that the highest P removal efficiency (75% P removal) corresponds
308 to pH = 7. A significant reduction in P uptake (ANOVA; $F_{2,6} = 441.2$, p value < 0.001)
309 was found with increasing pH value to pH = 8 (58% P removal) and pH = 9 (24 % P
310 removal). Different P adsorption efficiencies are reported in the literature for neutral pH
311 conditions. A lower P reduction (23%), but the same decreasing tendency with pH, was
312 found by Yao et al. (2014) when using ammonium-functionalized magnetic chitosan.
313 Filipkowska et al. (2014) found a 40 % of P reduction when using glutaraldehyde cross-
314 linked chitosan. Dixon (1984) reported a high P removal (85 %) by Fe_3O_4 that de-
315 creased with raising pH. Contrary to the results for MCMs, P adsorption efficiency by
316 bare carbonyl Fe microparticles is not pH dependent in the pH range (6-10) leading to a
317 high P reduction (80% P removal) whatever the pH (de Vicente et al., 2010). These re-

318 sults were explained by de Vicente et al. (2010) because of specific adsorption onto the
319 particles. It is worthy to highlight that initial P concentrations used by some of the men-
320 tioned authors were much higher than those used in this work.

321 Our results point out that pH dependency of the P adsorption onto MCMs is due to the
322 surface charge of the adsorbent and speciation of the adsorbate. MCMs are positively
323 charged in the pH range of 7-9 but the magnitude of charge decreases with pH (see elec-
324 trophoretic mobility data in Fig. 4). Electrophoretic mobility measurements seem to be
325 related to the P removal efficiency of the particles. Huang et al. (2010) also reported
326 positively charged magnetic chitosan nanoparticles in pure water. At lower pH, amine
327 groups of chitosan are more easily protonated than at higher pH values (Crini and Ba-
328 dot, 2008). At low pHs, P exists as both anions, dyhydrogen phosphate (H_2PO_4^-) and
329 hydrogen phosphate (HPO_4^{2-}) (Eliaz and Sridh, 2008) thus leading to the conclusion that
330 P adsorption onto MCMs is dominated by electrostatic interactions.

331 *3.4 P maximum adsorption capacity*

332 The adsorption data of the different adsorbents used in this study were fitted to Lang-
333 muir isotherm (Foo and Hameed, 2010):

$$334 \quad q = \frac{K_L M_L C_e}{1 + K_L C_e} \quad [3]$$

335 where K_L is an adsorption constant related to energy of adsorption (L g^{-1}) and M_L is an
336 empirical saturation constant that represents the maximum adsorption capacity (mg g^{-1})
337 (Foo and Hameed, 2010; Wong et al., 2004).

338 In the three cases, the Langmuir model showed a satisfactory fit to the experimental
339 data. In the case of MCMs and CMs (Fig. 5), at low phosphate concentrations, the ad-

340 sorption increased when increasing concentration but tended to reach a constant value at
341 higher phosphate concentrations in accordance to the model (Biswas et al., 2008) . Al-
342 though the same adsorption mechanism could be applied to Fe₃O₄, the saturation value
343 was reached very quickly compared to MCMs and CMs (Fig. 5). Table 1 shows the best
344 fitting parameters for Fe₃O₄, MCMs and CMs, and the corresponding correlation coeffi-
345 cients. Note that for comparative purposes, in these experiments, the magnetite concen-
346 tration was the same (constant) in Fe₃O₄ and MCMs suspensions. Also, the chitosan
347 concentration in CMs and MCMs suspensions was kept the same. The highest maxi-
348 mum P adsorption capacity (M_L) was reported by MCMs (4.84 mg g⁻¹) followed by
349 CMs (2.41 mg g⁻¹) and Fe₃O₄ (2.27 mg g⁻¹). Table 1 also summarizes maximum P ad-
350 sorption capacities of different adsorbents of interest found in the literature. P adsorp-
351 tion capacity values of Phoslock® (Haghseresht, 2005; Zamparas et al., 2012), alum (de
352 Vicente et al., 2008) or some clays such as Zenit/Fe (Zamparas et al., 2012) and clinop-
353 tilolite (Sakadevan and Bavor, 1998) are closer to values obtained for MCMs. On the
354 contrary, synthetic zeolite HUD (Onyango et al., 2007) and CFH-12 (Lyngsie et al.,
355 2014) present much higher P sorption capacities compared to MCMs. Isotherm data
356 pointed out a P removal efficiency by MCMs higher than 45 % for concentrations rang-
357 ing from 10 to 160 µmol P L⁻¹ when using a concentration of 0.72 g MCMs L⁻¹. Then, it
358 can be stated that MCMs would be potentially able to remove 60 % of P from water
359 column of hypertrophic water bodies whose average water column TP have been deter-
360 mined to be higher than 100 µg L⁻¹ (3.23 µmol P L⁻¹) (Nürnberg, 1996).

361 Differences in P adsorption capacity of Fe₃O₄, MCMs and CMs can be explained by the
362 different surface characteristics of the systems. The low P adsorption capacity found for
363 Fe₃O₄ is in the range of that found by Daou et al. (2007) (although at a different pH
364 value; pH = 3) who reported a maximum P adsorption capacity of 5.2 mg P g⁻¹ (Table 1)

365 when using a positively charged Fe_3O_4 with higher surface area (40 nm average particle
366 size). However, a much higher P adsorption capacity is also reported in the literature for
367 the same pH conditions to our study. de Vicente et al. (2010) found that negatively
368 charged Fe_3O_4 (90 nm) was able to adsorb a maximum of $27.15 \text{ mg P g}^{-1}$ (Table 1). At
369 $\text{pH} = 7$, our Fe_3O_4 exhibits a negative surface charge (electrophoretic mobility $-2.7 \pm$
370 $0.45 \mu\text{m cm V}^{-1} \text{ s}^{-1}$) which suggests that P adsorption mechanism is driven by specific
371 interactions (and not by electrostatic interactions). The comparatively low P adsorption
372 capacity found in our Fe_3O_4 is explained by the organic layer (supported by TGA and
373 FT-IR analysis) covering Fe_3O_4 surface that may difficult P adsorption onto hydroxyl
374 functional groups present on its surface (Antelo et al., 2005; Sun et al., 1998).

375 CMs adsorption capacity reported in this work is low compared to other studies employ-
376 ing cross-linked chitosan composites with the same pH conditions as the cases of Filip-
377 kowska et al. (2014) and Sowmya and Meenakshi (2014) who reported a P maximum
378 adsorption capacity of 58.5 mg g^{-1} and 108.2 mg g^{-1} , respectively (Table 1). Synthesized
379 CMs showed a slight negative surface charge as indicated by electrophoretic mobility ($-$
380 $0.84 \pm 0.65 \mu\text{m cm V}^{-1} \text{ s}^{-1}$) similarly to other cross-linked chitosan composites (Yao et
381 al., 2014). Indeed, the point of zero charge (pzc) for most chitosan particles has been
382 previously reported to range between 6.2 and 6.7 (Chatterjee et al., 2005; Elwakeel,
383 2009). P adsorption onto CMs is thought to be due to physical adsorption or intraparti-
384 cle diffusion through the chains network of the particle (Crini and Badot, 2008).

385 It is noteworthy that MCMs are positively charged despite of the fact that Fe_3O_4 and
386 CMs are negatively charged at $\text{pH} = 7$. This is expected because the embedded magnet-
387 ite grains interfere in the cross-linking process. The reason is that chitosan surface
388 charge is determined by protonation/deprotonation of its surface functional groups

389 (amine and hydroxyl groups) depending on pH (Pillai et al., 2009; Reddy and Lee,
390 2013). During the synthesis process in acidic media, aldehyde groups of glutaraldehyde
391 mainly react with protonated amine and hydroxyl groups (Crini and Badot, 2008). As
392 suggested in previous works, a higher extent of cross-linking reduces particle size and
393 surface zeta potential due to condensation of polymer chains and reduction of positively
394 charged free amino groups, respectively (Crini and Badot, 2008; He et al., 1999; Rodri-
395 gues et al., 2012). In our case, the larger particle size of MCMs (compared to CMs)
396 suggests a lower degree of cross-linking that is expected and attributed to the physical
397 interference of magnetite in the Schiff's base reaction giving a net positive charge to the
398 hybrid particles.

399 Reuse of MCMs is essential for their practical use and cost effectiveness. In this con-
400 text, a conventional protocol that works well in the case of iron oxides consists in wash-
401 ing the particles with a concentrated NaOH solution. With this in mind, after a typical
402 adsorption experiment, the MCMs particles were washed twice with 1M NaOH and 3
403 times with 3 mM NaHCO₃ using magnetic separation. This particular protocol although
404 successful for magnetite and carbonyl Fe particles (de Vicente et al., 2010) failed in the
405 case of MCMs (the P adsorption capacity of reused particles was very low). Degrada-
406 tion of the adsorbent may be another important issue for practical applications. How-
407 ever, chitosan solubilisation is not likely to occur in natural environments because
408 strong acidic conditions are required (Crini and Badot, 2008). Nevertheless, the cross-
409 linking level in MCMs may definitely have an effect and its impact in the resulting
410 chemical stability and mechanical resistance of the microparticles are open issues for
411 future work.

412 **4. Conclusions**

413 We propose a novel magnetic adsorbent for P removal from the upper sediment layers.
414 It consists on very low density hybrid multigrain microparticles prepared by a reverse-
415 phase suspension cross-linking technique. The microparticles are constituted by well
416 dispersed magnetite nanocores within a spherical chitosan microparticle (approx. 10 %
417 w/w) that is biocompatible and non toxic. These particles do exhibit a sufficient P ad-
418 sorption capacity ($M_L = 4.84 \text{ mg g}^{-1}$) to be used in eutrophic systems at conventional pH
419 levels. The mechanism behind the adsorption is dual: electrostatic for the case of chito-
420 san matrix and surface specific for the case of magnetite grains. This provides versatile
421 adsorption properties toward cation species as well. Because of their large size and
422 magnetite loading, these hybrid microparticles can be easily separated using magneto-
423 phoresis.

424 Apart from their versatile adsorption properties, the major advantage of these particles
425 is that, once settled from the water column, they have the ability to remain in the upper
426 layers of the sediment where the target sedimentary P pool in eutrophic lakes (P mobile)
427 concentrates. This is due to their much lower particle density compared to other mag-
428 netic adsorbents previously proposed for lake restoration. Another interesting aspect of
429 the synthesized microparticles is the low toxicity for aquatic biota due to their high par-
430 ticle size. Further investigation about MCMs reutilization is still needed in order to
431 make these particles cost-effective as P adsorbents.

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References

- Agnihotri, S.A., Aminabhavi, T.M., 2004. Controlled release of clozapine through chitosan microparticles prepared by a novel method. *J. Control. Release* 96, 245–259. doi:10.1016/j.jconrel.2004.01.025.
- Antelo, J., Avena, M., Fiol, S., López, R., Arce, F., 2005. Effects of pH and ionic strength on the adsorption of phosphate and arsenate at the goethite-water interface. *J. Colloid Interface Sci.* 285, 476–486. doi:10.1016/j.jcis.2004.12.032.
- Baumann, J., Köser, J., Arndt, D., Filser, J., 2014. The coating makes the difference: Acute effects of iron oxide nanoparticles on *Daphnia magna*. *Sci. Total Environ.* 484, 176–184. doi:10.1016/j.scitotenv.2014.03.023.
- Biswas, B.K., Inoue, K., Ghimire, K.N., Harada, H., Ohto, K., Kawakita, H., 2008. Removal and recovery of phosphorus from water by means of adsorption onto orange waste gel loaded with zirconium. *Bioresour. Technol.* 99, 8685–8690. doi:10.1016/j.biortech.2008.04.015.
- Cai, W., Wan, J., 2007. Facile synthesis of superparamagnetic magnetite nanoparticles in liquid polyols. *J. Colloid Interface Sci.* 305, 366–370. doi:10.1016/j.jcis.2006.10.023
- Carpenter, S.R., 2005. Eutrophication of aquatic ecosystems: bistability and soil phosphorus. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10002–10005. doi:10.1073/pnas.0503959102.
- Chatterjee, S., Chatterjee, S., Chatterjee, B.P., Das, A.R., Guha, A.K., 2005. Adsorption of a model anionic dye, eosin Y, from aqueous solution by chitosan hydrobeads.

J. Colloid Interface Sci. 288, 30–35. doi:10.1016/j.jcis.2005.02.055.

Chen, X.G., Park, H.J., 2003. Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. *Carbohydr. Polym.* 53, 355–359. doi:10.1016/S0144-8617(03)00051-1.

Choi, J., Chung, J., Lee, W., Kim, J.O., 2016. Phosphorous adsorption on synthesized magnetite in wastewater. *J. Ind. Eng. Chem.* 34, 198–203. doi:10.1016/j.jiec.2015.11.008.

Cooke, G.D., Welch, E.B., Peterson, S.A., Nichols, S.A., 2005. Restoration and management of lakes and reservoirs, third edition, Taylor and Francis Group, Boca Raton, Florida.

Crane, R.A., Scott, T.B., 2012. Nanoscale zero-valent iron: Future prospects for an emerging water treatment technology. *J. Hazard. Mater.* 211-212, 112–125. doi:10.1016/j.jhazmat.2011.11.073.

Crini, G., Badot, P.M., 2008. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. *Prog. Polym. Sci.* 33, 399–447. doi:10.1016/j.progpolymsci.2007.11.001.

Daou, T.J., Begin-Colin, S., Grenche, J.M., Thomas, F., Derory, A., Bernhardt, P., Legar, P., Pourroy, G., 2007. Phosphate adsorption properties of magnetite-based nanoparticles. *Chem. Mater.* 19, 4494–4505. doi:10.1021/cm071046v.

de Vicente, I., Huang, P., Andersen, F., Jensen, H.S., 2008. Phosphate adsorption by fresh and aged aluminum hydroxide. Consequences for lake restoration.

Environ. Sci. Technol. 42, 6650–6655. doi:10.1021/es800503s.

de Vicente, I., Merino-Martos, A., Cruz-Pizarro, L., de Vicente, J., 2010. On the use of magnetic nano and microparticles for lake restoration. *J. Hazard. Mater.* 181, 375–381. doi:10.1016/j.jhazmat.2010.05.020.

de-Bashan, L.E., Bashan, Y., 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003). *Water Res.* 38, 4222–4246. doi:10.1016/j.watres.2004.07.014.

Dittrich, M., Gabriel, O., Rutzen, C., Koschel, R., 2011. Lake restoration by hypolimnetic $\text{Ca}(\text{OH})_2$ treatment: Impact on phosphorus sedimentation and release from sediment. *Sci. Total Environ.* 409, 1504–1515. doi:10.1016/j.scitotenv.2011.01.006.

Dixon, D.R., 1984. Colour and turbidity removal with reusable magnetite particles-VII. A colloid chemistry study of the effect of inorganic ions on the efficiency of clarification. *Water Res.* 18, 529–534. doi:10.1016/0043-1354(84)90199-4.

Egemose, S., Wauer, G., Kleeberg, A., 2009. Resuspension behaviour of aluminium treated lake sediments: effects of ageing and pH. *Hydrobiologia* 2, 1–15. doi:10.1007/s10750-009-9949-8.

Eliaz, N., Sridh, T.M., 2008. Electrocrystallization of hydroxyapatite and its dependence on solution conditions. *Cryst. Growth Des.* 8, 3965–3977. doi:10.1021/cg800016h.

Elwakeel, K.Z., 2009. Removal of Reactive Black 5 from aqueous solutions using magnetic chitosan resins. *J. Hazard. Mater.* 167, 383–392.

doi:10.1016/j.jhazmat.2009.01.051.

Filipkowska, U., Jóźwiak, T., Szymczyk, P., 2014. Application of cross-linked chitosan for phosphate removal from aqueous solutions. *Prog. Chem. Appl. Chitin Its Deriv.* 19, 5–14. doi:10.15259/PCACD.19.01.

Foo, K.Y., Hameed, B.H., 2010. Insights into the modeling of adsorption isotherm systems. *Chem. Eng. J.* 156, 2–10. doi:10.1016/j.cej.2009.09.013.

Funes, A., de Vicente, J., Cruz-Pizarro, L., Álvarez-Manzaneda, I., de Vicente, I., 2016. Magnetic microparticles as a new tool for lake restoration: A microcosm experiment for evaluating the impact on phosphorus fluxes and sedimentary phosphorus pools. *Water Res.* 89, 366–374. doi:10.1016/j.watres.2015.11.067.

Funes, A., de Vicente, J., Cruz-Pizarro, L., de Vicente, I., 2014. The influence of pH on manganese removal by magnetic microparticles in solution. *Water Res.* 53, 110–122. doi:10.1016/j.watres.2014.01.029.

Gibbs, M.M., Hickey, C.W., Özkundakci, D., 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: Sediment incubations. *Hydrobiologia* 658, 253–275. doi:10.1007/s10750-010-0477-3.

Gómez-Pastora, J., Bringas, E., Ortiz, I., 2014. Recent progress and future challenges on the use of high performance magnetic nano-adsorbents in environmental applications. *Chem. Eng. J.* 256, 187–204. doi:10.1016/j.cej.2014.06.119.

Haghseresht, F., 2005. A revolution in phosphorus removal, Phoslock Water Solution Ltd. <http://www.phoslock.com.au> p. 21.

- He, P., Davis, S.S., Illum, L., 1999. Chitosan microspheres prepared by spray drying. *Int. J. Pharm.* 187, 53–65. doi: 10.1016/S0378-5173(99)00125-8.
- Huang, H.Y., Shieh, Y.T., Shih, C.M., Twu, Y.K., 2010. Magnetic chitosan/iron (II, III) oxide nanoparticles prepared by spray-drying. *Carbohydr. Polym.* 81, 906–910. doi:10.1016/j.carbpol.2010.04.003.
- Huber, D.L., 2005. Synthesis, properties, and applications of iron nanoparticles. *Small* 1, 482–501. doi:10.1002/sml.200500006.
- Iyengar, S.J., Joy, M., Ghosh, C.K., Dey, S., Kotnala, R.K., Ghosh, S., 2014. Magnetic, X-ray and Mössbauer studies on magnetite/maghemite core–shell nanostructures fabricated through an aqueous route. *RSC Adv.* 4, 64919–64929. doi:10.1039/C4RA11283K.
- Jiang, D.S., Long, S.Y., Huang, J., Xiao, H.Y., Zhou, J.Y., 2005. Immobilization of *Pycnopus sanguineus* laccase on magnetic chitosan microspheres. *Biochem. Eng. J.* 25, 15–23. doi:10.1016/j.bej.2005.03.007.
- Kalkan, N.A., Aksoy, S., Aksoy, E.A., Hasirci, N., 2012. Adsorption of reactive yellow 145 onto chitosan coated magnetite nanoparticles. *J. Appl. Polym. Sci.* 124, 576–584. doi: 10.1002/app.34986.
- Keller, A.A., Garner, K., Miller, R.J., Lenihan, H.S., 2012. Toxicity of nano-zero valent iron to freshwater and marine organisms. *PLoS One* 7, 1–10. doi:10.1371/journal.pone.0043983.
- Kumar, S., Dutta, J., Dutta, P.K., 2009. Preparation and characterization of N-heterocyclic chitosan derivative based gels for biomedical applications. *Int. J.*

Biol. Macromol. 45, 330–337. doi:10.1016/j.ijbiomac.2009.08.002.

Kumar, S., Koh, J., 2012. Physicochemical, optical and biological activity of chitosan-chromone derivative for biomedical applications. *Int. J. Mol. Sci.* 13, 6103–6116. doi:10.3390/ijms13056102.

Kyzas, G.Z., Deliyanni, E.A., 2013. Mercury(II) removal with modified magnetic chitosan adsorbents. *Molecules* 18, 6193–6214. doi:10.3390/molecules18066193.

Lai, L., Xie, Q., Chi, L., Gu, W., Wu, D., 2016. Adsorption of phosphate from water by easily separable Fe₃O₄@SiO₂ core/shell magnetic nanoparticles functionalized with hydrous lanthanum oxide. *J. Colloid Interface Sci.* 465, 76–82. doi:10.1016/j.jcis.2015.11.043.

Li, B., Shan, C.-L., Zhou, Q., Fang, Y., Wang, Y.-L., Xu, F., Han, L.-R., Ibrahim, M., Guo, L.-B., Xie, G.-L., Sun, G.-C., 2013. Synthesis, characterization, and antibacterial activity of cross-linked chitosan-glutaraldehyde. *Mar. Drugs* 11, 1534–1552. doi:10.3390/md11051534.

Liu, J., Sun, Z., Deng, Y., Zou, Y., Li, C., Guo, X., Xiong, L., Gao, Y., Li, F., Zhao, D., 2009a. Highly water-dispersible biocompatible magnetite particles with low cytotoxicity stabilized by citrate groups. *Angew. Chemie - Int. Ed.* 48, 5875–5879. doi:10.1002/anie.200901566.

Liu, X., Hu, Q., Fang, Z., Zhang, X., Zhang, B., 2009b. Magnetic chitosan nanocomposites: A useful recyclable tool for heavy metal ion removal. *Langmuir* 25, 3–8. doi:10.1021/la802754t.

- Long, F., Gong, J.L., Zeng, G.M., Chen, L., Wang, X.Y., Deng, J.H., Niu, Q.Y., Zhang, H.Y., Zhang, X.R., 2011. Removal of phosphate from aqueous solution by magnetic Fe-Zr binary oxide. *Chem. Eng. J.* 171, 448–455. doi:10.1016/j.cej.2011.03.102.
- Lovern, S.B., Klaper, R., 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ. Toxicol. Chem.* 25, 1132–1137. doi:10.1897/05-278r.1.
- Lu, A.H., Salabas, E.L., Schüth, F., 2007. Magnetic nanoparticles: Synthesis, protection, functionalization, and application. *Angew. Chemie - Int. Ed.* 46, 1222–1244. doi:10.1002/anie.200602866.
- Lyngsie, G., Penn, C.J., Hansen, H.C.B., Borggaard, O.K., 2014. Phosphate sorption by three potential filter materials as assessed by isothermal titration calorimetry. *J. Environ. Manage.* 143, 26–33. doi:10.1016/j.jenvman.2014.04.010.
- Massart, R., 1981. Preparation of aqueous magnetic liquids in alkaline and acidic media. *IEEE Trans. Magn.* 17, 1247-1248. doi: 10.1109/TMAG.1981.1061188.
- Mills, P., Snabre, P., 1994. Settling of a suspension of hard spheres. *Europhys. Lett.* 25, 651–656. doi:10.1209/0295-5075/25/9/003.
- Monier, M., Ayad, D.M., Abdel-Latif, D.A., 2012. Adsorption of Cu(II), Cd(II) and Ni(II) ions by cross-linked magnetic chitosan-2-aminopyridine glyoxal Schiff's base. *Colloids Surf., B.* 94, 250–258. doi:10.1016/j.colsurfb.2012.01.051.
- Monteiro, O.A.C., Airoidi, C., 1999. Some studies of crosslinking chitosan-glutaraldehyde interaction in a homogeneous system. *Int. J. Biol. Macromol.* 26,

119–128. doi:10.1016/S0141-8130(99)00068-9.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* 27, 31–36. doi:10.1016/S0003-2670(00)88444-5.

Neto, C.G.T., Giacometti, J.A., Job, A.E., Ferreira, F.C., Fonseca, J.L.C., Pereira, M.R., 2005. Thermal analysis of chitosan based networks. *Carbohydr. Polym.* 62, 97–103. doi:10.1016/j.carbpol.2005.02.022.

Nürnberg, G.K., 1996. Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. *Lake Reserv. Manag.* 12, 432–447. doi:10.1080/07438149609354283.

Oberdörster, E., 2004. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Perspect.* 112, 1058–1062. doi:10.1289/ehp.7021.

Onyango, M.S., Kuchar, D., Kubota, M., Matsuda, H., 2007. Adsorptive removal of phosphate ions from aqueous solution using synthetic zeolite. *Ind. Eng. Chem. Res.* 46, 894–900. doi:10.1021/ie060742m.

Philippova, O., Barabanova, A., Molchanov, V., Khokhlov, A., 2011. Magnetic polymer beads: Recent trends and developments in synthetic design and applications. *Eur. Polym. J.* 47, 542–559. doi:10.1016/j.eurpolymj.2010.11.006.

Pillai, C.K.S., Paul, W., Sharma, C.P., 2009. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog. Polym. Sci.* 34, 641–678. doi:10.1016/j.progpolymsci.2009.04.001.

- Reddy, D.H.K., Lee, S.M., 2013. Application of magnetic chitosan composites for the removal of toxic metal and dyes from aqueous solutions. *Adv. Colloid Interface Sci.* 201-202, 68–93. doi:10.1016/j.cis.2013.10.002.
- Reitzel, K., Hansen, J., Andersen, F., Hansen, K.S., Jensen, H.S., 2005. Lake restoration by dosing aluminum relative to mobile phosphorus in the sediment. *Environ. Sci. Technol.* 39, 4134–4140. doi:10.1021/es0485964.
- Robb, M., Greenop, B., Goss, Z., Douglas, G., Adeney, J., 2003. Application of PhoslockTM, an innovative phosphorus binding clay to two Western Australian waterways: Preliminary findings. *Hydrobiologia* 494, 237–243. doi:10.1023/A:1025478618611.
- Rodrigues, S., da Costa, A.M.R., Grenha, A., 2012. Chitosan/carrageenan nanoparticles : Effect of cross-linking with tripolyphosphate and charge ratios tripolyphosphate and charge ratios. *Carbohydr. Polym.* 89, 282–289. doi:10.1016/j.carbpol.2012.03.010.
- Sakadevan, K., Bavor, H.J., 1998. Phosphate adsorption characteristics of soils , slags and zeolite to be used as substrates in constructed wetland systems. *Wat. Res.* 32, 393–399.
- Shaikh, A.M.H., Dixit, S.G., 1992. Removal of phosphate from waters by precipitation and high gradient magnetic separation. *Water Res.* 26, 845–852. doi:10.1016/0043-1354(92)90016-W.
- Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506-509, 135–145.

doi:10.1023/B:HYDR.0000008611.12704.dd.

Søndergaard, M., Kristensen, P., Jeppesen, E., 1992. Phosphorus release from resuspended sediment in the shallow and wind-exposed Lake Arreso, Denmark. *Hydrobiologia* 228, 91–99.

Sowmya, A., Meenakshi, S., 2014. Effective removal of nitrate and phosphate anions from aqueous solutions using functionalised chitosan beads. *Desalin. Water Treat.* 52, 2583–2593. doi:10.1080/19443994.2013.798842.

Sugimoto, T., Matijević, E., 1980. Formation of uniform spherical magnetite particles by crystallization from ferrous hydroxide gels. *J. Colloid Interface Sci.* 74, 227–243. doi:10.1016/0021-9797(80)90187-3.

Sun, Z., Su, F., Forsling, W., Samskog, P., 1998. Surface characteristics of magnetite in aqueous suspension. *J. Colloid Interface Sci.* 197, 151–9. doi:10.1006/jcis.1997.5239.

Tang, S.C.N., Lo, I.M.C., 2013. Magnetic nanoparticles: Essential factors for sustainable environmental applications. *Water Res.* 47, 2613–2632. doi:10.1016/j.watres.2013.02.039.

Tian, Y., Yu, B., Li, X., Li, K., 2011. Facile solvothermal synthesis of monodisperse Fe₃O₄ nanocrystals with precise size control of one nanometre as potential MRI contrast agents. *J. Mater. Chem.* 21, 2476. doi:10.1039/c0jm02913k.

Wong, Y.C., Szeto, Y.S., Cheung, W.H., McKay, G., 2004. Adsorption of acid dyes on chitosan - Equilibrium isotherm analyses. *Process Biochem.* 39, 693–702. doi:10.1016/S0032-9592(03)00152-3.

- Wulff-Pérez, M., Torcello-Gómez, A., Gálvez-Ruíz, M.J., Martín-Rodríguez, A., 2009. Stability of emulsions for parenteral feeding: Preparation and characterization of o/w nanoemulsions with natural oils and Pluronic f68 as surfactant. *Food Hydrocoll.* 23, 1096–1102. doi:10.1016/j.foodhyd.2008.09.017.
- Yao, W., Rao, P., Lo, I.M.C., Zhang, W., Zheng, W., 2014. Preparation of cross-linked magnetic chitosan with quaternary ammonium and its application for Cr(VI) and P(V) removal. *J. Environ. Sci. (China)* 26, 2379–2386. doi:10.1016/j.jes.2014.03.005.
- Yavuz, C.T., Mayo, J.T., Yu, W.W., Prakash, A., Falkner, J.C., Yean, S., Cong, L., Shipley, H.J., Kan, A., Tomson, M., Natelson, D., Colvin, V.L., 2006. Low-field magnetic separation of monodisperse Fe₃O₄ nanocrystals. *Science* 314, 964–967.
- Zamparas, M., Gianni, A., Stathi, P., Deligiannakis, Y., Zacharias, I., 2012. Removal of phosphate from natural waters using innovative modified bentonites. *Appl. Clay Sci.* 62-63, 101–106. doi:10.1016/j.clay.2012.04.020.
- Zamparas, M., Zacharias, I., 2014. Restoration of eutrophic freshwater by managing internal nutrient loads. A review. *Sci. Total Environ.* 496, 551–562. doi:10.1016/j.scitotenv.2014.07.076.
- Zhang, L.Y., Zhu, X.J., Sun, H.W., Chi, G.R., Xu, J.X., Sun, Y.L., 2010. Control synthesis of magnetic Fe₃O₄-chitosan nanoparticles under UV irradiation in aqueous system. *Curr. Appl. Phys.* 10, 828–833. doi:10.1016/j.cap.2009.10.002.
- Zhang, W., Shen, F., Hong, R., 2011. Solvothermal synthesis of magnetic Fe₃O₄ microparticles via self-assembly of Fe₃O₄ nanoparticles. *Particuology* 9, 179–

186. doi:10.1016/j.partic.2010.07.025.

Zhou, Z.H., Xue, J.M., Wang, J., Chan, H.S.O., Yu, T., Shen, Z.X., 2002. NiFe₂O₄ nanoparticles formed in situ in silica matrix by mechanical activation. *J. Appl. Phys.* 91, 6015–6020. doi:10.1063/1.1462853.

Zhu, X., Wang, J., Zhang, X., Chang, Y., Chen, Y., 2010. Trophic transfer of TiO₂ nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. *Chemosphere*, 79, 928-933.

Figures

Fig. 1. SEM micrographs of (a) MCMs and (b) cross-sectioned MCMs.

Fig. 2. Magnetic hysteresis curve of (a) Fe_3O_4 and (b) MCMs.

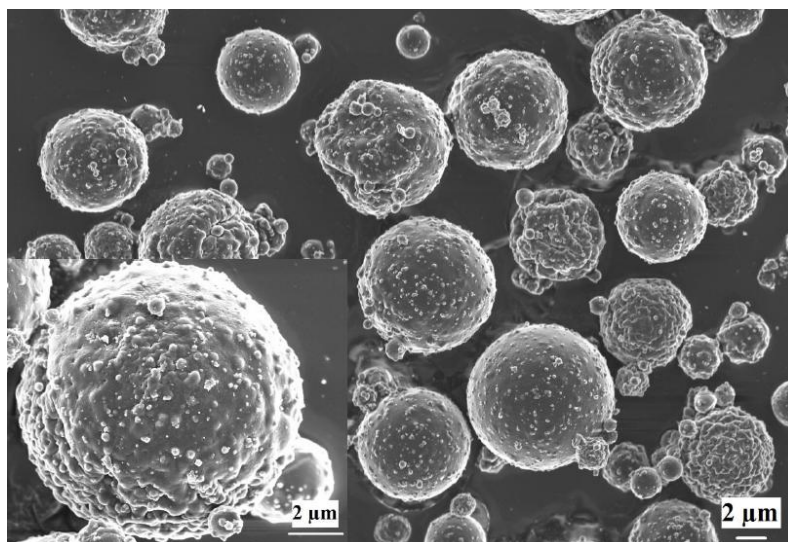
Fig. 3. Sedimentation ratio of carbonyl Fe microparticles (closed squares), and MCMs (open circles). H = sediment front determined at a constant value of transmission (5 %). H_0 = initial sample height (at time zero). Concentration: 0.72 g L^{-1} of carbonyl Fe particles/MCMs. pH = 7.

Fig. 4. Effect of pH on P removal efficiency (bars) and on electrophoretic mobility (square symbols) of MCMs. Initial concentrations: $0.72 \text{ g MCMs L}^{-1}$ and 0.01 mM P . Standard deviation is represented by vertical error bars.

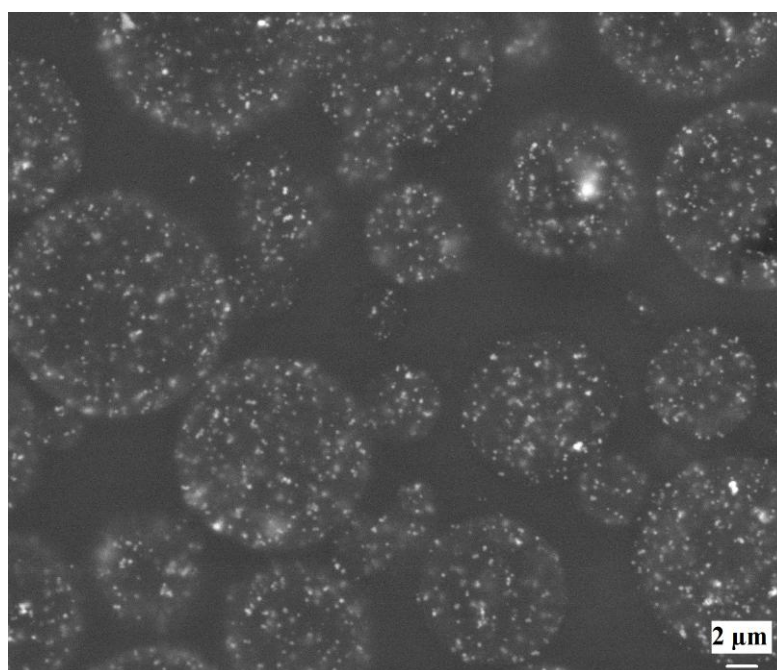
Fig. 5. Adsorption isotherm for Fe_3O_4 (open circles), MCMs (closed squares) and CMs (open triangles) at pH 7. Initial concentrations: $0.1 \text{ g Fe}_3\text{O}_4 \text{ L}^{-1}$, $0.72 \text{ g MCMs L}^{-1}$, $0.62 \text{ g CMs L}^{-1}$ and $0.01\text{-}0.16 \text{ mM P}$. Standard deviation is represented by vertical error bars.

Fig. 1

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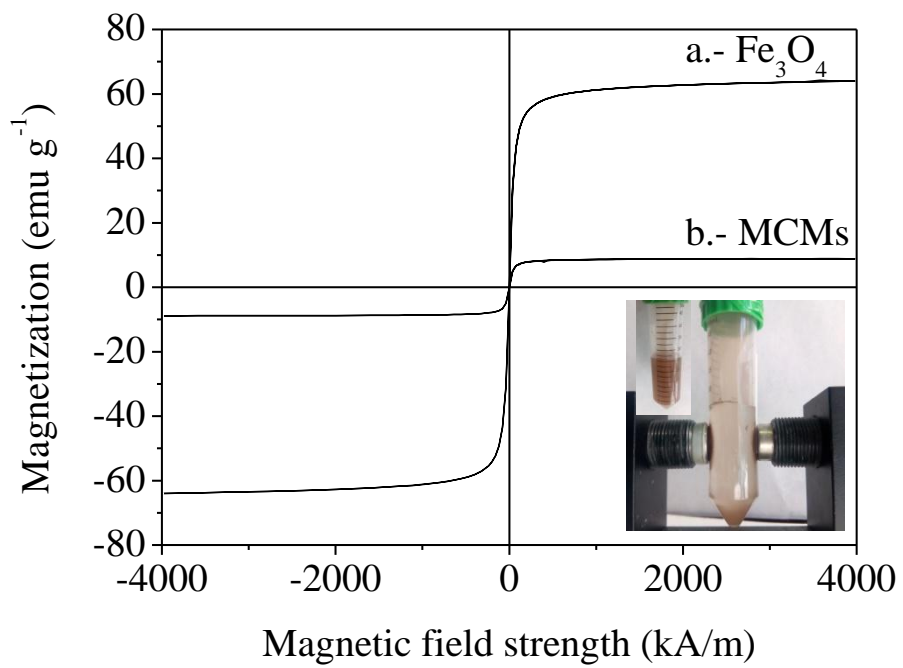


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1 **Fig. 2**

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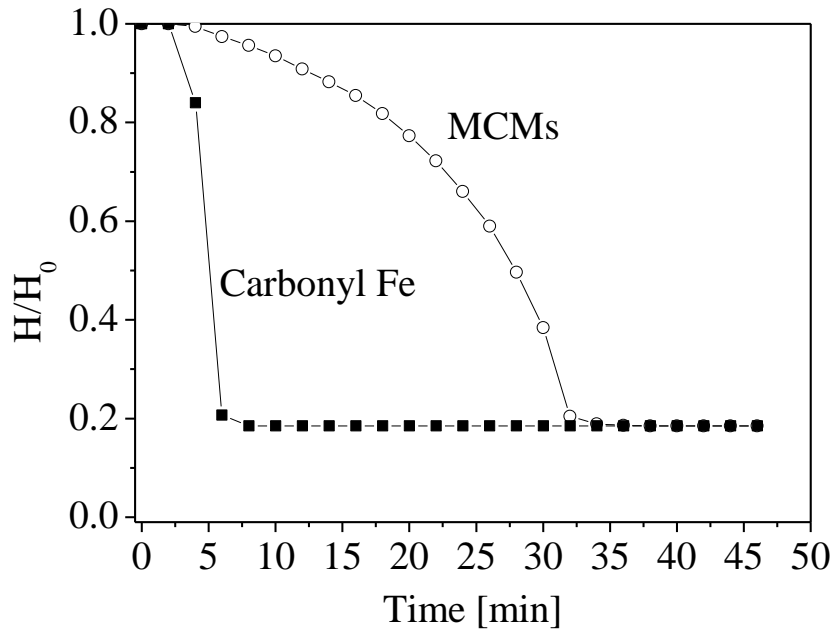


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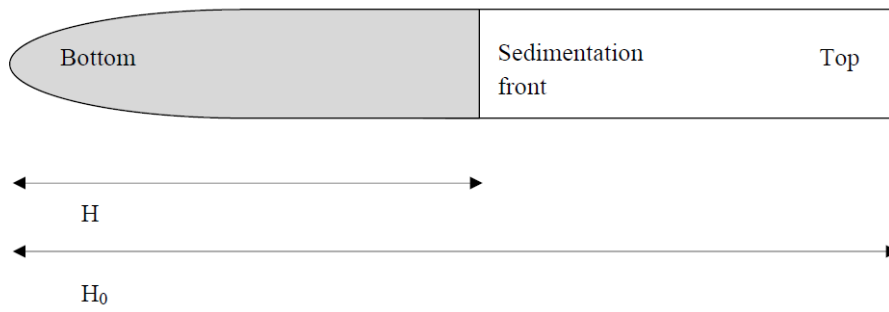
5 **Fig. 3**

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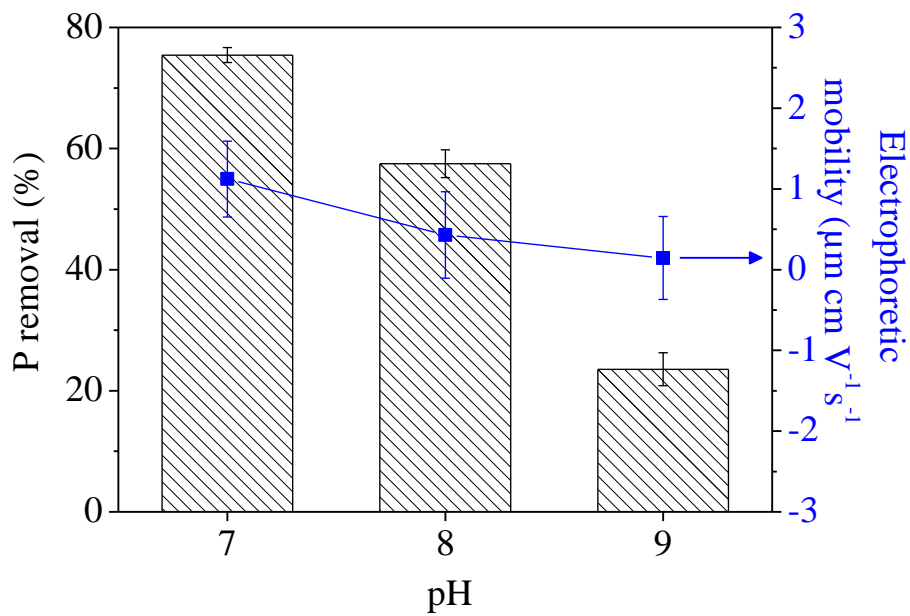
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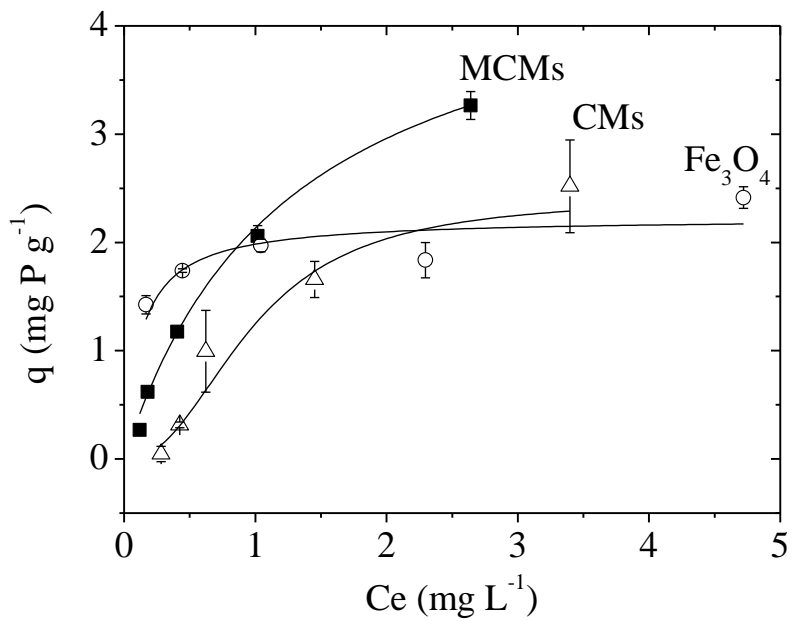
11 **Fig. 4**



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14 **Fig. 5**



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