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REMOVAL OF WATERBORNE MICROORGANISMS BY FILTRATION USING CLAY-POLYMER COMPLEXES

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Abstract

Clay-polymer composites were designed for use in filtration processes for disinfection during the course of water purification. The composites were formed by sorption of polymers based on starch modified with quaternary ammonium ethers onto the negatively charged clay mineral bentonite. The performance of the clay-polymer complexes in removal of bacteria was strongly dependent on the conformation adopted by the polycation on the clay surface, the charge density of the polycation itself and the ratio between the concentrations of clay and polymer used during the sorption process. The antimicrobial effect exerted by the clay-polymer system was due to the cationic monomers adsorbed on the clay surface, which resulted in a positive surface potential of the complexes and charge reversal. Clay-polymer complexes were more toxic to bacteria than the polymers alone. Filtration employing our optimal clay-polymer composite yielded 100 % removal of bacteria after the passage of 3 L, whereas an equivalent filter with granular activated carbon (GAC) hardly yielded removal of bacteria after 0.5 L. Regeneration of clay-polymer complexes saturated with bacteria was demonstrated. Modeling of the filtration processes permitted to optimize the design of filters and estimation of-experimental conditions for purifying large water volumes in short periods.

Key words: water purification, filtration, clay-polymer complexes, bacteria, modeling

1. Introduction

Disinfection processes are crucial in water treatment utilities. Disinfection is traditionally performed in drinking water treatment plants (WTPs) by chlorination, which reduces significantly pathogens in water but may pose a serious risk to human health due to formation of disinfection by-products (DBPs) [1]. The presence of a minute amount of natural organic matter in chlorinated waters can induce the formation of trihalomethanes (THMS) and haloacetic acids (HAAS), which are carcinogenic. The use of chloramination instead reduces the formation of these chemicals but leads to the formation of nitrosamines [2].

Ozone is also a very powerful disinfectant able to remove a wide range of microorganisms including those resistant to other oxidative means, such as chlorination. However, it is a very unstable molecule, which decomposes very quickly. Studies have shown undesired effects after ozonation, such as formation of nitrosamines [3] and cyanogen halides [4]. Advanced oxidation processes based on the attack of the target molecules by hydroxyl radicals generated by UV irradiation in the presence of oxidants, such as ozone, H_2O_2 or TiO_2, are capable of degrading very efficiently numerous prions [5].

Disinfection processes are greatly improved in combination with other water treatment processes such as filtration technologies [6-8]. Depth filtration is incorporated in the vast majority of WTPs, and helps to reduce the loading of waterborne pathogens by physical sorption or entrapment in addition to removal of particles to which they are associated. Moreover, it may be effective for removal of DBP precursors. Membrane

filtration processes are mostly advantageous for very stringent water quality standards because of their high operational costs [9].

One of the most widely-used materials in column filtration is GAC; however, this material has very poor performance for removal of pathogens. Therefore, present research is focusing on GAC modification and the synthesis of new composite materials to be used as media for microorganism [10-14]. An alternative is the use of polymer-based composites due to the antimicrobial properties exerted by cationic polymers [15]. These composites are of particular interest when dealing with water soluble polymers, where surface anchorage is needed for their preparation. Tashiro et al. [16] prepared polymers based on polystyrene supported in alumina granules, which presented high adsorption rate constants in *Escherichia coli*'s removal. Madkour et al. [17] eliminated *E. coli* and *Staphylococcus aureus* from water by using glass surfaces grafted with poly(butylmethacrylate)-*co*-poly(boc-aminoethyl methacrylate). However, ~~only few~~ studies showing the potential use of polymer based composites in water filtration processes for removal of microorganisms are scarce [18].

Clay-polymer composites can be designed by adsorption of cationic polymers onto negatively charged clay mineral platelets. The driving forces for polymer sorption are the translational entropic gain due to removal of water molecules and counter ions from the clay surface, and the electrostatic attraction between the polymer and the clay surface [19]. Adsorption of certain polycations on clay minerals was considered irreversible [20]. The use of clay-polymer composites in the removal of microorganisms from water by filtration has not been thoroughly studied yet. In the current study, we aimed at (i) designing clay-polymer composites with antibacterial properties based on the sorption of cationic starches onto a commercial bentonite; (ii) elucidating the mechanisms and factors involved in the development of toxicity of the new composites;

(iii) testing their efficiency in the removal of the pathogenic enteroindicator *E. coli* by filtration; and (iv) analysis of the kinetics of filtration for generating estimates for a variety of situations, e.g., upscaling. The polymers used were cationic starches which are widely used as additives in paper-making, textile and cosmetic industry.

2. **Materials and Methods**.

2.1. Materials.

The cationic starches employed were a gift from Penford Co. (Centennial, CO) and are based on the reaction of hydroxyl groups of pristine starch with 3-chloro-2-hydroxypropyltrimethylammonium (chemical structure in Fig. 1). Three types of polymers were studied differing in their degree of substitution (DS). All of them are commercial: Topcat L-98 (DS=0.22) (denoted hereafter as P1); Topcat L-95 (DS=0.15) (denoted as P2) and Penbond 1000 (DS=0.05) (P3). Their charge densities (CD) were determined to be respectively, 1.19 meq/g for P1, 0.846 meq/g for P2, and 0.29 meq/g for P3. A commercial Na-bentonite (Bentonil A, CEC 0.8 $mmol_c/g$) was kindly supplied from Süd-Chemie Spain. Granular activated carbon (GAC) (NUSORB GC60, 12x30 mesh) was purchased from NUCON International, Inc. (Columbus, OH).

The bacterial strain *E. coli* was purchased from the Spanish Type Culture Collection (CECT): The Luria-Bertani growth medium and the Agar for the microbial assays were supplied by Merck (Darmstadt, Germany). The LIVE/DEAD BacLight Bacterial Viability kits were obtained from Life Technologies (Carlsbad, CA, USA).

2.2 *Sorption of polymers onto the clay.*

Sorption isotherms of the polymers onto the commercial bentonite were carried out by mixing 15 mL of polymer solutions (0-40 g/L) with 24 mg of clay. The clay

concentration was 1.6 g/L. After shaking for 24 h at 20ºC, the suspensions were centrifuged at 12000 g for 10 min, the supernatants were discarded and the pellets were dry-frozen. The sorbed amount of polymer was determined by elemental C analysis.

The zeta potential (ξ) of the polymer-clay complexes obtained after sorption was measured by redispersing with distilled water at a concentration of 1.6 g/L. The samples were allowed to equilibrate for 1h and few milliliters of dispersion were measured using a Zetasizer Nanosystem (Malvern Instruments, Southborough, MA).

X-ray diffraction of oriented samples on glass slides was also measured using a Philips X'Pert diffractometer (model Anton Paar HTK) at low and higher angles on a Siemens diffractometer (model D5000). The samples were prepared from the paste obtained after centrifugation of the polymer-clay suspensions of the adsorption experiments.

Several clay polymer complexes were prepared for their study in the next sections. In general, clay powder was added to a polymer solution; the suspension was shaken for 24 h and centrifuged; the pellet was dry-frozen yielding the clay-polymer composite. A nomenclature for the different clay-polymer composites was introduced where the first two characters indicate the type of polymer, the following number denotes the polymer concentration added in g/L and the last number the clay concentration used in g/L.

2.3 Determination of bactericidal effects of clay-polymer composites.

Escherichia coli were incubated for 24h at 37ºC in Luria-Bertani nutrient broth, and a bacteria suspension with a 10^5 CFU/mL concentration was prepared. Clay-polymer complexes were added to this suspension in centrifuge tubes at a 1.5:100 solid: water ratio. This ratio was chosen after preliminary trials to see differences in the bactericidal activity of the prepared clay-polymer complexes. After 1 h incubation at 25ºC, the suspensions were centrifuged at 1000 rpm for 10 min at 4ºC, and 0.1 mL of the

suspensions were removed and mixed with 0.9 mL of sterile distilled water, and then successive decimal serial dilutions were prepared. From the suspensions and successive dilutions, the surviving bacteria were counted on nutrient media by the spread-plate method and expressed as colony forming units (CFU) per milliliter of sample. The plates were incubated at 37ºC and the colonies were counted after 24 h. The counting was done in four replicates every time. The limit of quantification (LOQ) for bacteria analysis with the spread-plate method is 10 CFU/mL. If no colonies are recovered, the limit of detection (LOD) is reported to be <10 CFU/ml for a 1:10 dilution according to the ASTM International.

In a parallel experiment, the deactivation of the cells after interaction with the clay complexes was examined by using a LIVE/DEAD stain methodology. Briefly, 1 mL of the suspension was incubated in darkness for 15 min with 4 μL of a mixture of propidium iodide and the SYTO 9 dye. After centrifugation, the pellets were mounted on slides and examined with a Zeiss Axioskop epifluorescence microscope at 40x magnification counting the dead and live cells on the clay-polymer surfaces by emission of red and green light, respectively. Three sections were examined for each slide and the results were expressed as percent of dead cells over the total counted. Preliminary experiments showed no difference in the counting after replacing the supernatant with distilled water, vortexed for 1 min and centrifuged again.

2.4. Removal of microorganisms by filtration.

In Experiment 1, column filter experiments were performed with 50/1 (w/w) mixture of quartz sand and clay-polymer complexes or GAC. Glass columns of 21 cm in length and 2 cm in diameter and with a porous plate at the bottom were filled with 73.5 g of thin quartz sand mixed with 1.5 g of clay-polymer complexes or GAC. The active

sorbent layer was 13 cm length. Glass wool (0.35 g) was placed on both ends of the column to prevent exit of the sand from the column. The pore volume of the column was 12.9 mL. The column was connected to a peristaltic pump and saturated at a constant flow rate of 7 mL/min with distilled water (equivalent to a flow velocity of 1.3 m/h). Then, an *E. coli* suspension of 10^5 CFU/mL prepared freshly from a stock solution, under continuous stirring started to pass-by. Preliminary experiments showed that the suspension was stable when staying 3 d at room temperature. Experiment 2 was analogously performed but only with the active material P1/10/4.25 at a flow rate of 4 mL/min (or 0.76 m/h). In another set of experiments (Experiment 3), two columns were connected in series and aliquots were taken at the exit of each column. The flow rate was constant at 7 mL/min (or 1.3 m/h).

2.4.1 Regeneration

In Experiment 1, after the columns were saturated by passing a suspension containing 10^5 CFU/mL of *E.coli*, two procedures were assayed for regeneration: (i) 1L of a 0.1 M HCl solution was passed through the column at a flow rate of 0.6 mL/min, and washed after that with 1 L of distilled water; (ii) idem but a commercial solution of sodium hypochlorite (2% w:v) was used instead of the acid. A new similar suspension including 10^5 CFU/mL *E. coli* was passed through the filters. The choice of these reagents was to examine the competitive effect of protons to detach the negatively-charged bacteria interacting with the positively charged clay-polymer composites; and the use of a common reagent used in WWT for regeneration of membranes through oxidation of pollutants.

2.5. Analysis of the kinetics of filtration.

In this analysis, the adsorption and convection phenomena occurring in the filter are modeled as in Nir et al. [21] (details in Supporting Information). The parameters employed in the calculation are: molar concentration of adsorbing sites is Ro, C1 (M^{-1} min^{-1}), rate constant of forward adsorption), and D1 (min^{-1}), rate constant of desorption)

2.6. Statistical analysis

Statistical analyses were carried out using the JMP IN software package (SAS Institute Inc., NC, USA). One way analysis of variance (F-test ANOVA, p = 0.05) was used to check the influence of each factor. All experiments were done at least three times, each treatment with four replicates.

The statistical criteria employed for simulation and prediction of certain experimental results of filtration by the calculations according to Eq(1) were the values of R^2 and RMSE, the Root Mean Square Error, defined by

$$RMSE= (\sum(Y_{Ci} - Yexpi)^2/(n-2))^{0.5} \quad (2)$$

in which n is the number of data points (we used averages of triplicates), and Y_{Ci} and Yexpi are the calculated and experimental values of percent removal. The term (n- 2) in Eq(2) is due to using 2 adjustable parameters.

3. Results and Discussion.

3.1. Clay-polymer composites.

Fig. 2 shows the sorption isotherms of the polymers and zeta potential of the composites formed. The sorption behavior of the polymers showed an initial steep increase as expected from strong Coulombic interactions with the negatively charged clay surface. Further sorption yielded positive values of the zeta potential, reaching higher values for P1 (about +30 mV), followed by P2 (~+25 mV) and P3 (~+15 mV).

214 Based on the degree of substitution, the calculated charge density (CD) was 1.19 meq/g
215 of polymer for P1; 0.846 meq/g of polymer for P2 and 0.29 meq/g of polymer for P3.
216 Charge neutralization at the external surface was observed at a loading of 0.3 g of
217 polymer/g clay for P1 and P2, and 0.8 g of polymer/g clay for P3; thus accounting for
218 0.36, 0.25 and 0.23 meq/g clay for P1, P2 and P3, respectively. These lower values for
219 the point of zero charge (p.z.c.) than the CEC of the clay can be explained by the
220 extending positive segments, or by the high screening of the clay surface by non-
221 charged segments of the polycation after sorption [22].
222 The loading of polymer on the clay was highest for P3 reaching a sorption plateau at
223 1.73 g polymer/g clay, whereas lower amounts were observed for P2 and P1. The
224 polymer loading on the clay can be rationalized on the basis of CD, which determines
225 the strength of the polyelectrolyte-surface interactions. For low CD polyelectrolytes
226 such as P3, the lower amount of strong electrostatic interaction with the clay surface
227 increases the importance of the steric repulsion of the uncharged portion of the polymer
228 backbone between adjoining charged segments neutralized by the clay surface [23].
229 Therefore, these uncharged portions are extending into solution in the form of loops and
230 tails, resulting in both thicker adsorbed layer and higher loadings. In contrast, the
231 stronger interactions with the clay surface for polycations with high CD as would be for
232 P1 and P2, result in a flat conformation of the polymer molecule on the clay surface,
233 i.e., the area occupied by one single molecule is higher, yielding lower adsorbed
234 amounts.
235 Evidence for these conformations of the polycation molecules on the clay surface was
236 supported by XRD (Fig. 3). The clay itself showed the typical diffraction of
237 montmorillonite (M), but some impurities were detected due to illite (I), kaolinite (K),
238 quartz (Q), and feldspars (F). The peak at 1.37 nm is typical of montmorillonite in its

239 sodium form with two layers of water of hydration. Sorption of polymers P1 and P2 at
240 high loading showed in both cases an increase in the basal spacing up to 1.42 nm (Fig.
241 3b). This value is in agreement with polymer sorption by forming a flat layer, because
242 the thickness of dextran polymers which are analogous to starch polymers (mainly
243 constituted by glucopyranose molecules) is about 0.5 nm [19], which by addition of the
244 thickness of a clay platelet (0.96 nm) gives a basal spacing around 1.46 nm. Similarly,
245 basal spacings of about 1.5 nm have been reported for other polymers adsorbing on clay
246 minerals as flat layers [24].
247 With P3, the XRD analysis of the clay-polymer complexes showed the absence of the
248 diffraction peak associated to montmorillonite (Fig. 3c) even at low diffraction angles
249 (not shown), indicating a loop-and-train conformation on the clay surface that yielded
250 basal spacing out of the range of XRD detection. This is also taken as evidence of
251 exfoliation of the clay platelets after interaction with the polycation molecules [25].
252 Clay-composites were prepared at lower polymer/clay ratios aiming at increasing the
253 amount of polymer adsorbed. A clay concentration of 4.25 g/L was used; the amounts
254 of polymer added were 10 g/L for P1 and P3, and 5 g/L for P2. The estimated sorbed
255 amounts for these ratios were quite close for P2 and P1 (0.40 and 0.45 g polymer/g clay,
256 respectively), and 1.12 g polymer/ g clay for P3. These values are in accord with the
257 predictions by theoretical sorption models where the total adsorbed amount of the solute
258 should increase when raising the clay concentration but the amount adsorbed per clay
259 platelet decreases [26]. However, polymer sorption increased instead of decreasing as
260 expected, from 0.57 to 0.80 g polymer/g clay for P1 and from 0.35 to 0.68 g polymer/g
261 clay for P2. The total adsorbed amount for P3 slightly decreased (from 1.78 to 1.72 g
262 polymer/g clay).Our tentative explanation for the above pattern is that increasing the

clay concentration decreases the polymer fraction available for self-aggregation and enhances the polymer sorption onto the clay.

3.2. **Determination of bactericidal effects of clay-polymer composites.**

The bactericidal effects of the clay-polymer complexes on bacteria in suspension was examined as a function of the amount of polymer sorbed by the clay, the conformation of the polymer on the clay platelets, and the surface charge characteristics of the complex. (Table 1).

Initially, 10^5 CFU/mL of *E. coli* were present in solution in the bactericidal tests. As a control, raw clay was used and no bactericidal effect was noticed (data not shown); whereas no free bacteria were observed when using the complexes P1/10/4.25 and P2/5/4.25. This was not only a function of the positive external surface potential which is needed for adhesion of the bacteria, because the sorption of bacteria by the complex P1/5/1.6 was less efficient-despite its identical zeta potential.

The concentration of cationic monomers of the polymer over that needed for inducing charge reversal was a critical parameter. The P1/5/1.6 complex reduced only two orders of magnitude the initial amount of added bacteria, whereas an increase by +0.33 $mmol_c/g$ polymer in the case of P2/5/4.25 (lines 3,4 in Table 1), enabled to reach the critical concentration needed for complete removal.

The influence of the conformation adopted by the polycation on the removal of bacteria was also examined. The activity of the complex P2/1.5/1.6 with a layer flat conformation of the polycation on the clay surface, was one order of magnitude larger than that of the complex P3/10/4.25, where the polymer molecules had a loop-and-train conformation. Both complexes exhibited-the same z-potential, but the amount of adsorbed polymer and the corresponding monomer concentration exceeding that needed

for reaching the zero zeta potential of the clay was about 5-fold larger for the complex P3/10/4.25. In accord, its capacity for removal of bacteria from water was lower, which was due to the fact that in a loop-and-train conformation the positive charges of polymer segments extending into the solution are also partly screened by hydrophobic segments impeding a closer interaction of the cationic groups with the bacterial cell surface.

The target site of quaternary ammonium polymers was reported to be the cytoplasmic membrane [27]. The positive charge of the polycation apparently impairs the stability of the cell wall of negatively charged bacteria, and also the outer membrane in the Gram negative type. After penetration through the cell wall, the polycation is attracted to the cytoplasmic membrane, increasing its permeability, and yielding bactericidal effect by cell lysis [28]. Similarly to the bactericidal effect exhibited by the free polymers, it might be also interpreted that the polymer -clay composites have bactericidal effect on bacteria. The results in Table 1, which demonstrate a reduction in the number of bacteria in suspension by the presence of a polymer –clay composite can also be simply interpreted by adsorption of the bacteria (which are characterized by a negative external surface) on the positively charged composites. However, Table 1 demonstrates different removal efficiency of bacteria by composites with the same zeta potential. In addition to removal of bacteria from water, the results in Table 1 also indicate that cell death rates on the clay-polymer complexes were high (approximately 90%) with the exception of the P3/10/4.25 complex. These data demonstrate the high sorption efficiency and bactericidal effect of the clay-polymer complexes based on polymers P1 and P2; as opposed to the P3-clay complexes, in which case both extent of bacteria adsorption and killing are about 10%.

In the other cases the percent of adsorbed bacteria exceeded 93% and the percent of dead cells exceeded 85% of the total.

The clay-polymer complexes were more bioactive than the polymers alone (Table S1, Supporting Information). The bactericidal effect was much lower from polymer solutions which included equivalent amounts to those of polymers sorbed in the clay-polymer complexes (Table 1). The larger concentration of cationic monomers onto the clay surface was high enough to reach lethal levels for the bacteria.

The explanation of the bactericidal mechanism caused by polymer-clay composites is still under discussion [29, 30]. Our results may point to a combined bactericidal effect of the surface potential and the corresponding amount of cationic monomers of the polymer exceeding that for inducing charge reversal of the clay. A positive surface potential on the clay complex promotes adherence of the bacteria followed by direct strong electrostatic interactions that would cause a segregation of negatively charged phospholipids from the cell membranes. A high cationic monomer concentration in the vicinity of phospholipid bilayers will introduce large-number of disrupting contact points in the bilayer continuity by intercalation of the hydrated bactericide groups between the negatively charged phospholipid headgroups, impeding their isolating function and further restoration, and yielding cell lysis.

3.3. **Filtration of *E. coli*.**

Fig. 4 shows a poor removal of bacteria by filters containing GAC relative to the clay-polymer based-filters, especially those containing the polymers P1 and P2. After 0.5 L, the GAC based-filter did not retain *E. coli*, whereas approximately 80% was retained with the filters containing P3-clay complex and no elution was detected with the other clay-polymer complexes. The bacteria retention in the filters containing the complex P3/10/4.25 was lower than in those based on P1- and P2-clay complexes, in agreement with its poor removal of bacteria in suspension (Table 1). The use of the P1/10/4.25

complex in the filter improved greatly retention of bacteria compared to P2/5/4.25, which is in agreement with its larger amount of cationic monomers exceeding the CEC on the clay surface, as revealed in the batch experiments. The emergence of minute amounts of bacteria was not detected after the passage-of 3 L, i.e., 155 pore volumes larger than with the complex P2/5/4.25. Therefore, the filters based on the complex P1/10/4.25 were the optimal ones for microorganism removal. The performance of filters under different operational parameters, such as the flow rate, and concentration of bacteria added, was investigated and modeled (Tables 2, 3). A comparison of the results in Table 2 (Experiment 3) with those in Table 3 (Experiment 2) demonstrates that a larger volume (5.3 L) could be purified (below the LOD) from bacteria in Experiment 2 than in the former (2.5 L) despite the larger number of bacteria per unit volume, ($5x10^5$ vs 1.2 10^5 per mL) in the latter case, corresponding to the condition that filtration was carried out at a smaller flow rate in Experiment 2.

3.3.1 Regeneration

A preliminary study of the feasibility of regeneration of the filter containing P1/10/4.25 showed complete regeneration by using either HCl or NaClO. At the end of filtration (Table 2) the fraction of removed bacteria was 70%, whereas after regeneration bacteria were only detected after passing 3L. At that stage the fractions of removed bacteria were 99.8 and 99.9% for the filters regenerated with HCl and NaClO respectively, versus an initial value of 99.7%. These regenerated filters followed the same pattern of bacterial removal as that of the newly used filters; for example, the removal percents were 96.5 and 98.0% after passing 3.4 L when using HCl- and NaClO-regenerated filters, respectively, whereas a similar value (95.6%) was obtained initially.

363 3.3.2. Modeling and capacity estimates

364 The fitting of the kinetics of filtration to the adsorption-convection model was adequate;
365 therefore, the calculated parameters can be used for prediction under different
366 operational parameters. It is of interest to note that the second filter in series in
367 Experiment 3 (Table 2) enabled to purify the water completely (i.e, below the LOD)
368 from bacteria after 8.4 L, i.e., at a 3-fold larger volume than that by the first filter alone.
369 In Table 3 complete removal of bacteria was achieved after the passage of 5.3 L through
370 the filter which included 1.5 g of complex. If the capacity of the filter is defined by the
371 volume which can be purified from bacteria to less than one particle per 100 mL (from
372 an initial value 5.2 $x10^5$ /ml in this case), then this result amounts to a capacity of 3.5 m^3
373 / kg of composite. Using the same parameters as in Table 3 we calculated the kinetics of
374 filtration for the case of a filter of length 1.6 m, at a flow velocity of 4.9 m/h , and filled
375 with a composite at a 1:5 w/w ratio between the composite and sand. The outcome for
376 the capacity in this case was 12 m^3 /kg of composite. For a filter filled exclusively with
377 the composite in a granular form no sand would be needed, and the calculated capacity
378 was 20 m^3/kg of composite. Furthermore, the success of regeneration implies that the
379 effective capacity can be significantly larger. The filtration results presented suggest
380 that it may be of interest to test the use of clay-polymer composites in removal of other
381 microorganisms and on a larger scale.

382 In view of the fact that the chosen polymer is relatively inexpensive (€ 9.7/kg) and the
383 clay is rather cheap (€ 0.16/kg), it can follow that the cost of materials of large scale
384 purification of drinking water which includes 100,000. bacteria per mL to less than 1
385 per 100 mL can be less than 0.5 € per cubic meter. In this calculation we tentatively
386 assumed that the preparation of a granulated composite will double the price of raw
387 materials. The success of regeneration by washing the filter with rather inexpensive

388 solutions (in particular HCl) will reduce this cost further. The next needed steps will be
389 granulation and test of upscaling.
390
391 **4. Conclusions**
392 The results indicate: (i) the most efficient removal of bacteria by filtration occurred
393 when the columns included the complex P1/10/4.25 as shown in Tables 2 and 3, in
394 agreement with Table 1 which gives bacterial removal and killing in batch experiments
395 and in accord with its larger amount of cationic monomers exceeding the CEC on the
396 clay surface in this complex; (ii) destabilization of bacterial membranes was more
397 efficient in the presence of the polymer-clay complexes than by the polymers alone; (iii)
398 calculations of filtration kinetics yielded adequate simulations and predictions and
399 estimates for upscale; (iv) preliminary experiments showed complete regeneration of
400 filters loaded by bacteria by using either HCl or NaClO. The outcome of the current
401 studies suggests that filtration by engineered polymer-clay composites can be a valid
402 technology for purification of water from bacteria, and perhaps from other
403 microorganisms.
404
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411

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412

413 **Figure captions**

414 Fig.1. Chemical basic structure of the cationic starches.

415 Fig. 2. Polymer adsorption (open symbols) and z-potential (full symbols) of the
416 complexes formed after sorption on bentonite of P1 (a), P2 (b) and P3 (c), as a function
417 of the added amount of the polymer.

418 Fig. 3. Fig. 3. X-ray diffraction of the bentonite (a) and polymer-clay complexes at the
419 maximal loading from the sorption isotherms: 0.9 and 0.6 g/ g clay for P1 and P2
420 respectively (b) and 1.73 g/g clay for P3 (c).

421 Fig. 4. Removal of *E. coli* by filtration with columns including GAC, or polymer-clay
422 complexes mixed with sand (1:100 w/w) (Experiment 1).

423

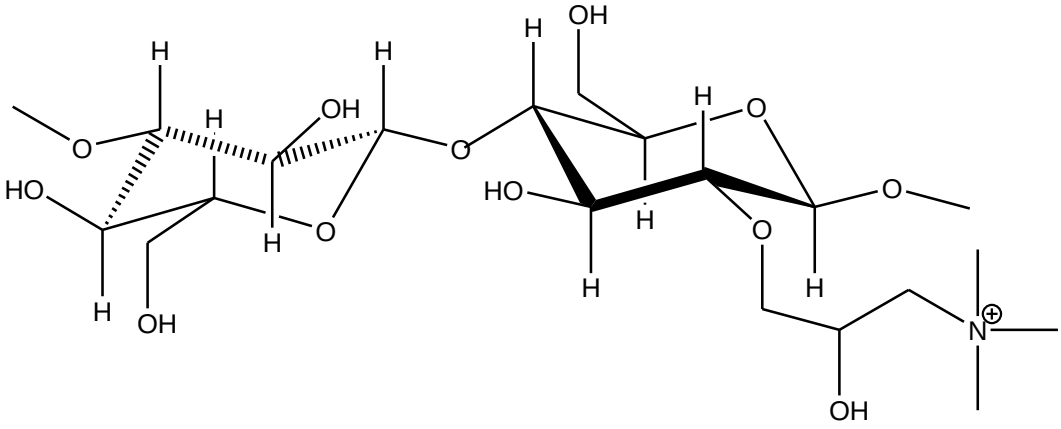


Figure 1

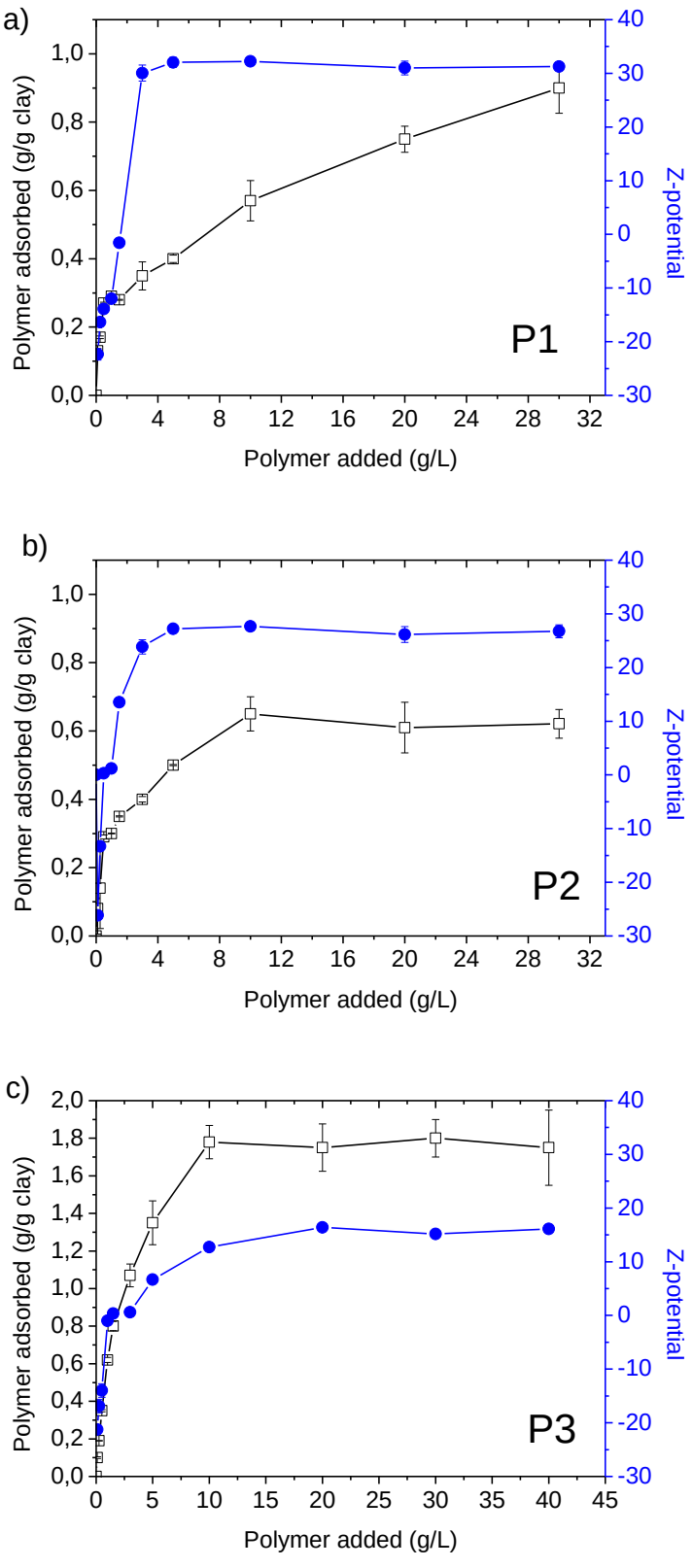
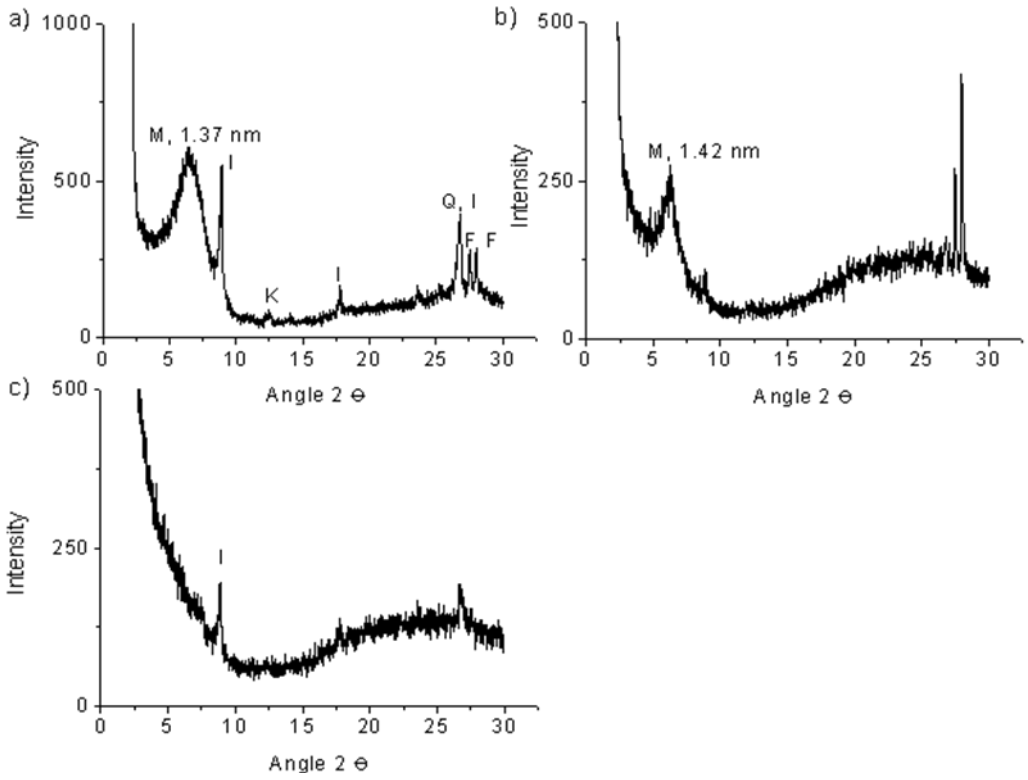

a)
Polymer adsorbed (g/g clay)
Z-potential
Polymer added (g/L)
P1
b)
Polymer adsorbed (g/g clay)
Z-potential
Polymer added (g/L)
P2
c)
Polymer adsorbed (g/g clay)
Z-potential
Polymer added (g/L)
P3

Figure 2



437

438 Figure 3

439

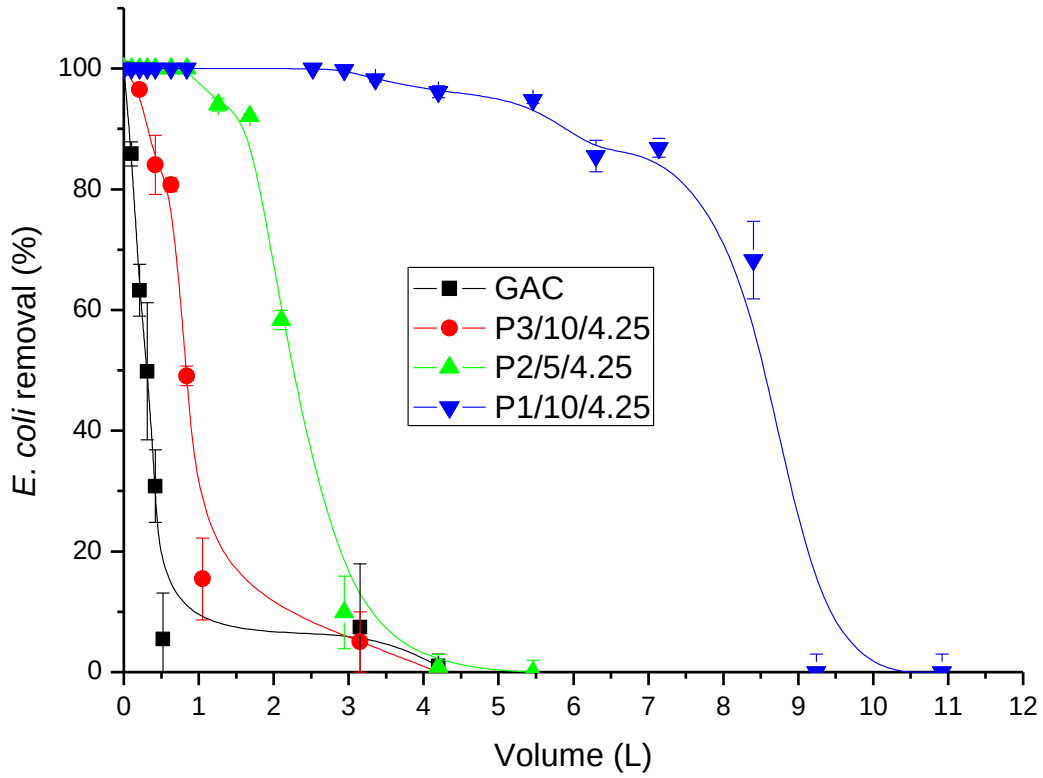

E. coli removal (%)
Volume (L)
GAC
P3/10/4.25
P2/5/4.25
P1/10/4.25
0
20
40
60
80
100
0
1
2
3
4
5
6
7
8
9
10
11
12

Figure 4

446 Table 1. Effect of polymer loading and surface charge of clay-polymer complexes on
447 bacterial adsorption in suspension, and toxicity to *E.coli* expressed as viable bacterial
448 cells (in brackets as log removal) and the percent of dead cells on the clay-polymer
449 surface after incubation. The initial *E. coli* concentration was 10^5 CFU/mL.

Clay-polymer complex[1]	Polymer sorbed (g/g clay)	Charge[3] (meq_c/g clay)	Z-Potential (mV)	*E. coli*,[2] CFU/mL	*Dead cells (% of the total)*[2]
P1/5/1.6	0.40±0.04	+0.12	31.0±1.3	$6.4x10^3$ (1.2) b	90.9a
P1/10/4.25	0.80±0.01	+0.60	28.7±4.8	0 (5)c	85.5a
P2/5/4.25	0.68±0.01	+0.32	26.7±4.1	0 (5)c	90.6a
P2/1.5/1.6	0.35±0.01	+0.04	13.6±0.7	$2.8x10^3$ (1.6) b	89.4a
P3/10/4.25	1.72±0.03	+0.27	12.7±0.3	$1.4x10^4$ (0.9) a	12.9b

450 1. Notation: polymer name/polymer concentration/clay concentration.
451 2. Means followed by the same letter indicate that either the toxicity exhibited by
452 the composite or the death rates were not significantly different according to
453 Student´s test at P=0.05.
454 3. Charge of sorbed polymer beyond the zero point of zeta potential.
455
456
457

458 Table 2. *E. coli* removal (%) from water after filtration using P1/10/4.25 complex
459 (Experiment 3). Experimental (Exp.) and calculated (Calc.) values. The initial *E. coli*
460 concentration was $1.2x10^5$ CFU/mL.[1,2]

	Removal (%)			
	Column 1		Column 2	
Volume (L)	Exp.	Calc.	Exp.	Calc.
0.4	100	99.5	100	100
0.8	100	99.2	100	100
2.5	100	97.1	100	99.9
2.9	99.7	96.4	100	99.9
3.4	98.2	95.3	100	99.8
4.2	95.6	93.5	100	99.7
5.5	94.7	89.8	100	99.5
6.3	85.5	86.9	100	99.4
7.1	85.7	83.6	100	99.2
8.4	72.7	78.1	100	98.7

461

462 1. The parameters used were $R_0 = 1.4x10^{-12}$ M , in which R_0 indicates the total molar
463 concentration for binding sites for the bacteria in the filter. $C_1 = 2.5x10^{12}$ $M^{-1}min^{-1}$, where C_1 is
464 the forward rate constant of binding of bacteria to the polymer clay composite, and $D_1=0.0026$
465 min^{-1} , where D_1 is the rate constant of dissociation of bound bacteria.
466 2. The statistical analysis of the results gave RMSE= 2.2 and $R^2= 0.881$.
467
468
469
470

Table 3. *E. coli* removal (%) from water after filtration using P1/10/4.25 complex (Experiment 2). Experimental (Exp.) and calculated (Calc.) values. The initial *E. coli* concentration was 5.2×10^5 CFU/mL. [1]

Volume (L)	Exp.	Calc.
5.3	100	99.9
5.8	99.7	99.7
6.2	99.6	99.4
7.2	97.2	98.8
8.2	96.4	97.8
9.6	94.9	95.0
11.5	87.1	86.3

1. The parameters used in the calculations were $R_0 = 1.4 \times 10^{-12}$ M; $C_1 = 3 \times 10^{12}$ $M^{-1}min^{-1}$, and $D_1 = 0.0012$. min^{-1}. The RMSE was 1.0 and R^2 was 0.925.

Supporting information

REMOVAL OF WATERBORNE MICROORGANISMS BY FILTRATION USING CLAY-POLYMER COMPLEXES

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Analysis of the kinetics of filtration.

In this analysis, the adsorption and convection phenomena occurring in the filter are described by Eq (1) as in Nir et al. [21]. A column of length L is filled with material whose initial molar concentration of adsorbing sites is Ro, whose concentration changes later to R(X,t). The beginning and end of the filter are at the coordinates X = 0 and X = L, respectively. We consider that the pollutant concentration at the inlet, Co, is constant, i.e., C(X,t) = Co, $X \leq 0$, where t denotes time.

The kinetic parameters are C1 (M^{-1} min^{-1}, rate constant of forward adsorption), D1 (min^{-1}, rate constant of desorption), v (flow velocity); α (≤ 1) denotes the degree of hysteresis, which was not considered in this case.

$$dC(X,t)/dt = -v \partial C/\partial X - C1 \cdot C(X,t)\, R(X,t) + \alpha\, D1\, (Ro - R(X,t)) \quad (1)$$

Table S1. Zeta potential and toxicity of polymer solutions equivalent to the polymer loading on the clay complexes used as in Table 1. Added *E. coli* concentration were 10^5 CFU/mL.

Polymer used	Conc. equivalent to complex	Z-potential (mV)	*E. coli*,[1] CFU/mL
P1	P1/5/1.6	29.3±1.0	$1.1x10^5$(0)a
P1	P1/10/4.25	25.6±2.9	$7.7x10^4$ (0.1)b
P2	P2/5/4.25	26.1±1.2	$1.6x10^5$(0)a
P2	P2/1.5/1.6	14.5±2.3	$2.8x10^5$(0)a
P3	P3/10/1.6	13.5±2.5	$3.5x10^5$(0)a

1. In brackets as log removal. Means followed by the same letter indicate that the toxicity exhibited by the composite was not significantly different according to Student´s test at P=0.05.