Filtration of microorganisms by 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 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.

Keywords: water purification; clay-polymer complexes; bacteria

Introduction

Disinfection processes are greatly improved in combination with other water treatment processes such as filtration technologies (Ho et al., 2012). Depth filtration is incorporated in the vast majority of Wastewater Treatment Plants, 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. One of the most widely-used materials in column filtration is GAC; however, this material has very poor performance for removal of pathogens. An alternative is the use of polymer-based composites due to the antimicrobial properties exerted by cationic polymers (Kenawy et al., 2007). Clay-polymer composites can be designed by adsorption of cationic polymers onto negatively charged clay mineral platelets. The use of clay-polymer composites in the removal of microorganisms from water by filtration has not been thoroughly studied yet.

The current study is 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; and (iii) testing their efficiency in the removal of the pathogenic enteroinicator E. coli by filtration;

Material and Methods

Preparation of clay-polymer complexes. A commercial bentonite (Bentonil, Clariant Int. Ltd.) was used. The solutions of three cationic starches with different degree of substitution (P1, DS 0.22; P2, DS 0.15 and P3, DS 0.05) were added to clay powder; the suspensions were 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.

**Determination of bactericidal effects of clay-polymer composites.** *Escherichia coli* were incubated for 24h at 37°C 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. After 1 h incubation at 25°C, the suspensions were centrifuged at 1000 rpm for 10 min at 4°C, and the bactericidal effect of the supernatant was measured by the by the counting spread-plate method. In a parallel experiment, the deactivation of the cells after interaction with the clay complexes was examined by using a LIVE/DEAD stain methodology.

**Removal of microorganisms by filtration.** 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. The pore volume of the column was 12.9 mL. Then, an *E. coli* suspension of $10^5$ CFU/mL started to pass-by at a flow velocity of 1.3 m/h.

**Results and Conclusions**

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

Several clay polymer/complexes were prepared differing in 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). In the complexes based on the polymers P1 and P2, the polymer molecule adopted a flat conformation on the clay surface as a consequence of the strong electrostatic interactions with the negatively charged clay surface; thus the area occupied by one single molecule is higher when compared to the polymer P3, yielding lower adsorbed amounts. On the contrary, with the P3-clay-based complexes, the lower amount of strong electrostatic interaction with the clay surface increases the importance of the steric repulsion of the uncharged portion of the polymer backbone between adjoining charged segments neutralized by the clay surface. Therefore, these uncharged portions are extending into solution in the form of loops and tails, resulting in both thicker adsorbed layer and higher loadings.

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

<table>
<thead>
<tr>
<th>Clay-polymer complex</th>
<th>Polymer sorbed (g/g clay)</th>
<th>Charge (meq/g clay)</th>
<th>Z-Potential (mV)</th>
<th><em>E. coli</em>, CFU/mL</th>
<th>Dead cells (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1/5/1.6</td>
<td>0.40±0.04</td>
<td>+0.12</td>
<td>31.0±1.3</td>
<td>$6.4\times10^3$ (1.2) b</td>
<td>90.9a</td>
</tr>
<tr>
<td>P1/10/4.25</td>
<td>0.80±0.01</td>
<td>+0.60</td>
<td>28.7±4.8</td>
<td>0 (5)c</td>
<td>85.5a</td>
</tr>
<tr>
<td>P2/5/4.25</td>
<td>0.68±0.01</td>
<td>+0.32</td>
<td>26.7±4.1</td>
<td>0 (5)c</td>
<td>90.6a</td>
</tr>
<tr>
<td>P2/1.5/1.6</td>
<td>0.35±0.01</td>
<td>+0.04</td>
<td>13.6±0.7</td>
<td>2.8$\times10^4$ (1.6) b</td>
<td>89.4a</td>
</tr>
<tr>
<td>P3/10/4.25</td>
<td>1.72±0.03</td>
<td>+0.27</td>
<td>12.7±0.3</td>
<td>1.4$\times10^4$ (0.9) a</td>
<td>12.9b</td>
</tr>
</tbody>
</table>

1. **Notation:** polymer name/polymer concentration/clay concentration.
2. **Means followed by the same letter indicate that either the toxicity exhibited by the composite or the death rates were not significantly different according to Student's test at $P=0.05$.
3. **Charge of sorbed polymer beyond the zero point of zeta potential**
After sorption of the polymer on the clay, charge neutralization of the negatively charged clay surface occurred at concentrations of cationic monomers lower than the cationic exchange capacity (CEC) of the clay (0.8 mmol_c g\(^{-1}\) clay), as a result of the high screening of the clay surface by non-charged segments of the polycation. The concentration of cationic monomers in excess over that needed for neutralization of the clay surface was determined and reported in Table 1.

The bactericidal effects of the clay-polymer complexes were also shown in Table 1. These effects were not only a function of the positive external surface potential of the clay-polymer complex 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. Clearly, the concentration of cationic monomers of the polypolymer 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 +0.32 mmol_c/g polymer in the case of P2/5/4.25, 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. This lower capacity for removal of bacteria from water with the complex P3/10/4.25 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 results in Table 1 may be interpreted as a reduction in the number of bacteria in suspension by the presence of a polymer—clay composite by adsorption of the bacteria (which are characterized by a negative external surface) on the positively charged composites; however, the LIVE/DEAD stain methodology showed that the cell death rates on the clay-polymer complexes were high (approximately 90%) with the exception of the P3/10/4.25 complex.

Filtration of E. coli.

Figure 1 showed 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.

![Graph showing E. coli removal](image)

**Figure 1.** Removal of *E. coli* by filtration with columns including GAC, or polymer-clay complexes mixed with sand (2:100 w/w).

**References**
