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Removal of bacteria and *Cryptosporidium* from water by micelle–montmorillonite complexes

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ABSTRACT

This study aimed at testing the application of nano-composites of micelle–montmorillonite for removal of bacteria and parasites from water. The suitability of these complexes for efficient adsorption of microorganisms was expected on account of their large surface areas, large excess of positive charge, and existence of large hydrophobic domains. Tests included removal from water of bacteria: Gram negative (*Escherichia coli* K-12), Gram positive (*Bacillus megaterium)*, and a protozoan parasite, *Cryptosporidium parvum*. Micelles of the organic cations Benzyldimethylhexadecylammonium (BDMHDA), or Octadecyltrimethylammonium complexed with the clay-mineral montmorillonite were shown to reduce by 3–6 orders of magnitude the numbers of microorganisms in water as tested in suspension and by filters (20 cm in length) packed with the complexes mixed with excess sand (100:1, w/w). Respiration tests demonstrated that *E. coli* K-12 cells adsorbed to micelle (BDMHDA)–montmorillonite complex lost their viability. Kinetics of filtration of *E. coli* by two filters in series was adequately simulated by a model which considers convection, adsorption. and desorption. Filtration of *E. coli* K-12 cells demonstrated that they stayed adsorbed and inactivated by the complex within the column filter without re-growth in water. These results suggest that filters including micelle–montmorillonite composites can secure the safety of drinking water in case of threatening increases in the number of pathogenic microorganisms in the water.

Keywords: Water treatment; Micelle-montmorillonite; Bacteria; *Cryptosporidium*; Adsorption; Filtration

1. Introduction

In the last decades, pollution of groundwater and surface water has become an environmental and economical hazard due to anthropogenic contaminants. Discharges of industrial wastes and sewage, and run-off from agricultural land have affected water quality [1].

One concern regarding water quality is minimizing the concentration of pathogenic microorganisms in

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water, thereby reducing the risk of an outbreak of waterborne diseases in humans or animals. A wide variety of enteric microbial pathogens may be found in wastewater [1]. A number of bacterial pathogens which cause diseases such as dysentery, typhoid, and gastroenteritis have been transmitted primarily through untreated sewage-irrigated vegetables [2].

The pathogenic waterborne protozoa group includes *Giardia lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica*. In untreated wastewater, *Giardia* concentrations have been reported to be as high as 3,375 cells/L [3]. *Cryptosporidium* oocysts have been detected in wastewater and drinking water sources. Water chlorination is inadequate for inactivation of oocysts of *Cryptosporidium* [4]. The species *C. parvum* is the main agent of human cryptosporidiosis. Transmission of the parasite occurs by the fecal-oral route, through the ingestion of oocysts that are shed with the feces of infected hosts. Immunocompetent individuals experience short-term gastroenteritis, while immunocompromised patients may suffer from chronic diarrhea [5]. Together with the resistance of oocysts to environmental conditions and chlorine [6], a large parasite reservoir and a low infective dose account for the risk of transmission by water. Numerous *Cryptosporidium* outbreaks have been described [7].

Chlorination has been the main strategy for the disinfection of drinking water and wastewater, by inactivating pathogenic microorganisms. Alternative technologies have been evaluated because of the increasing concern over undesirable production of trihalomethanes, due to chlorination at high doses, whereas reduced efficiency in eliminating some epidemic microorganisms occurs at low doses [8].

Alternative means of disinfection include chloramines, chlorine dioxides, ozone, and ultraviolet radiation, which also have several major disadvantages, such as the formation of carcinogenic by-products, residuals in treated water, and reactivation of microorganisms after exposure. Since an ideal disinfection technique has not yet been found, combinations of several techniques are being used [8].

In an attempt to enhance the adsorption of microorganisms to surfaces, Preston et al. [9] added cationic polymers to fiberglass filters to increase the adsorption efficiency of viruses to filters from large volumes of water and no effect of the pH value was observed. On the other hand, Lukasik et al. [10] proposed the use of columns packed with sand coated with ferric and aluminum hydroxides for the removal of microorganisms from water. Siedbrath et al. [11] described cleaning of secondary effluents by organoclays, powdered activated carbon, and $FeCl_3$.

We present a novel means for the removal of microorganisms from water using two types of micelle–montmorillonite sorbents. The micelle–montmorillonite system is a relatively new innovation in which the composite has a large surface area, a substantial fraction with a hydrophobic core, and is positively charged to a varying degree [12]. It was shown by X-ray diffraction, electron microscopy, and adsorption experiments that the material characteristics of the micelle–montmorillonite complex are different from those of an organo–montmorillonite complex, which is formed by adsorption of the same organic cation, Octadecyltrimethylammonium (ODTMA), as monomers. For instance, the anionic herbicide sulfometuron was almost completely adsorbed by the micelle–montmorillonite composite, but was hardly adsorbed by the organo–montmorillonite complex, despite its same composition [12]. The micelle–montmorillonite composite has already been proven useful in the removal of about 20 neutral and anionic pollutants [13–15]. Studies demonstrated that the release of the organic cations, ODTMA and Benzyldimethylhexadecylammonium (BDMHDA), from the complex during filtration of large volumes was minimal and could be reduced to the ppb level and lower by an addition of a layer of montmorillonite mixed with excess sand (150:1, w/w) [16], and unpublished results available upon request. We hypothesized that this system in addition to the removal of micropollutants could be powerful for the removal of pathogenic microorganisms from water. Enteric bacteria, viruses, and protozoan parasites carry a negative surface charge [17,18]. Therefore, this system could be useful for the removal of organic pollutants and pathogenic microorganisms from water.

The main aim of this article is to demonstrate that nano-composites of micelle-montmorillonite can be considered as efficient means for removal of bacteria and parasites from water by filtration. We investigated in batch and filtration experiments, the comparative removal from water of representative types of gram-negative and gram-positive bacteria and *C. parvum*.

2. Materials and methods

2.1. Materials

The montmorillonite used was Wyoming Na-montmorillonite SWy-2 (grain size 0.8–1.5 mm) obtained from the Source Montmorillonites Repository (Montmorillonite Minerals Society, Columbia, MO). Quartz sand was obtained from Negev Industrial Minerals (Israel). BDMHDA and ODTMA were purchased from

Fluka Chemie (Buchs, Switzerland) and Sigma–Aldrich (Sigma Chemical Co., St. Louis MO), respectively. Non-woven polypropylene geotextile filters were obtained from Markham Culverts Ltd., Papua New Guinea.

2.2. Cultivation and enumeration of bacterial strains

Escherichia coli K-12, and *Bacillus megaterium*, from the collection of the Department of Plant Pathology and Microbiology, were routinely grown in Luria Bertani medium (LB; Difco) at the appropriate temperature. Quantitative cell counting was performed by the plate dilution method.

E. coli (K-12) was maintained by a passage on fresh mFC selective culture. Isolated colonies were obtained after incubation for 20 h at 37˚C. Fresh culture of *E. coli* was obtained by the transfer of a colony to 5 ml of LB broth and incubation for 20 h at 37˚C. A culture of *E. coli* in the log phase was obtained by transferring the overnight culture to 50 ml of LB broth. An *E. coli* culture was obtained by incubation for 4–5 h, in a shaker water bath at 100 rpm. The *E. coli* culture was washed by pelleting at 3,000 g for 15 min and re-suspending the bacteria in phosphate-buffered saline (PBS). *E. coli* enumeration was accomplished by the membrane filtration method using LB agar.

B. megaterium spores were obtained by incubating a culture for 7 days at 37˚C in a shaking water bath. The *B. megaterium* culture was washed by pelleting at 3,000 g for 15 min and re-suspending the pellet in PBS. The percentage of the spores in the culture was determined by exposure to 70˚C for 15 min. *B. megaterium* enumeration was performed on typtone agar [19,20].

2.3. C. parvum *detection and enumeration*

C. parvum oocysts, Iowa strain, were obtained from Waterborne (Waterborne Co. Baton Rouge, LA). Staining with FITC-labeled antibodies and microscopic examination were performed as described in Method 1,623 (USEPA). A sample of 50 µl of oocysts of *C. parvum* was applied to a special slide obtained from Waterborne (Waterborne Co. Baton Rouge, LA). The sample was allowed to dry at room temperature, and then fixed with 50 µl of absolute methanol. After drying, 50 µl of FITC-labeled anti *C. parvum* monoclonal antibody was added and the samples were incubated for 30 min at room temperature. The samples were then washed, air-dried, and to each sample 50 µl of 1,4-diazobicyclo (2,2,2) octane (DAPCO) was added. The samples were enumerated using an Exiscope II epi- fluorescence microscope (Zeitz, Germany) at a magnification of 1000X.

2.4. Effect of BDMHDA on cell viability

The effect of BDMHDA on cell viability was analyzed by two different strategies: (A) BDMHDA (0.60 mM) was added to a fresh culture of *E. coli* K-12 diluted with LB to reach 10^6–10^7 CFU/ml. Samples were taken every 10 min and the bacteria were enumerated on LB agar; (B) Fresh growth cultures were diluted with LB to reach 10^3 CFU/ml and 100 µl aliquots was placed on LB-agar containing different concentrations of BDMHDA (0–0.60 mM); Plates were then incubated and the number of colonies was recorded daily.

2.5. Effect of micelle–montmorillonite complex on E. coli viability as measured by respiration

The micelle–montmorillonite complex was prepared in glass vials sealed with a rubber stopper. Different ratios between the BDMHDA–montmorillonite complex and additional sterilized montmorillonite or sand were examined. *E. coli* K-12 was grown on LB liquid medium. The cells were washed with 0.9% NaCl solution and diluted to OD_{600}=0.25 corresponding to a concentration of 10^7 cfu/ml.

One milliliter of the culture was added to each vial and the suspension was shaken for 30 min. Then, 100 µl of 10% glucose was added to each vial and the vials were sealed and incubated for 24 h at 30˚C. A sample of 1 ml air was taken from each vial and analyzed by gas chromatography (GC model 580, Gow-Mac Instruments Co; Bridgewater, NJ, USA, equipped with Poropak Q column and TCD detector).

The CO_2 concentration in the test samples was compared to the CO_2 concentration obtained from vials with air only (~374 ppm).

Attempts to test bacterial cell viability by a Biotium Inc. kit [21] were not possible, because the two fluorescent indicators in the kit, Etd-III, and DMAO were adsorbed by the BDMHDA–montmorillonite complex.

2.6. Preparation of the micelle–montmorillonite complex

The micelle–montmorillonite complex was prepared as described by Polubesova et al. [14] with slight modifications. The complex was prepared by stirring 12 mM of BDMHDA or ODTMA with 10 g/L montmorillonite for 48 h at room temperature. The suspension was centrifuged for 20 min at 15,000 g, the supernatant was discarded, and the complex was lyophilized.

2.7. Batch adsorption experiments

Batch experiments were performed in 125-ml Erlenmeyer flasks containing 0.2 g of micelle–montmorillonite complex and 20 ml of bacterial cell suspension

at a concentration of 10^5–10^7 CFU/ml. Samples were mixed in a rotary shaker for 30 min at 200 rpm at room temperature; then, centrifuged for 15 min at 3,000 g at 4°C. Supernatant samples were taken for immediate bacterial analysis. Microorganisms were also added to sand quartz and sand quartz-montmorillonite (without micelle-montmorillonite) as controls.

2.8. Column filter experiments

Column filters were filled with 650 g sand mixed with 6.5 g of micelle–montmorillonite complex (23 cm layer) in a column of 25-cm length and 5-cm diameter (a ratio of 1:100). The bottom of the column was covered by 2-cm layer of quartz sand (thoroughly washed by distilled water and dried at 105°C for 24 h). Non-woven polypropylene geotextile filters were placed at both ends of the column. The column was connected to a peristaltic pump and saturated with distilled water or tap water from the bottom (flow rate of 5 ml/min). The packed columns were dosed with water seeded with the tested bacteria or *C. parvum* at a concentration range of 10^4–10^6 CFU or oocyst/ml. The volume of the influent was 2 L, or as indicated. Microorganism concentration was monitored in the influent and effluent samples. Column experiments were also performed using two packed column filters placed in series.

The ability of *E. coli* to be released from the filter and grow was tested by taking out the contents of the central part of the filter and placing it on a sheath of sterile aluminum foil. One gram of material was agitated by Vortex in 10 ml PBS. The supernatant was plated in LB solid medium.

2.9. Theoretical analysis of kinetics of filtration

The model assumed that adsorption of microorganisms by the micelle–clay complex obeys the Langmuir equations which ignores interaction between adsorbing particles. Accordingly, the model assumes that the kinetics of filtration is controlled by convection and adsorption of the microorganisms by the surfaces of the micelle–clay complexes.

The adsorption and convection are described by Eq. (1) below; numerical solution was executed by a FORTRAN program [22]. A column of length L is filled with material whose initial molar concentration of adsorbing sites is R_o, 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, C_o, is constant, i.e. $C(X,t) = C_o$, $X \leqslant 0$, where t denotes time.

The kinetic parameters are C_1 ($M^{-1}\,min^{-1}$, rate constant of forward adsorption), D_1 (min^{-1}, rate constant of desorption), v (flow velocity); α (<1) denotes the degree of hysteresis, which was not considered in this case.

$$dC(X,t)/dt = -v\,\partial C/\partial X - C_1 \times C(X,t)R(X,t) + \alpha \times D_1(R_0 - R(X,t)) \quad (1)$$

2.10. Statistics

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 three 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 the Root Mean Square Error (RMSE), defined by

$$\mathrm{RMSE} = \left(\sum (Y_{C_i} - Y_{\exp_i})/(n-2)^{0.5}\right) \quad (2)$$

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

3. Results

As indicated, our main goal in this study was to test the efficiency of removal of microorganisms from water by means of adsorption by the micelle–clay complex. Clearly, efficient removal by adsorption implies filtration. However, in view of the fact that this is the first study which employs the micelle–clay complex for this purpose, we also explored several related issues, e.g. adsorption in suspension, bactericidal activity of the complex, and probed whether interaction of bacteria with the complex can result in their loss of viability.

3.1. Bactericidal activity of BDMHDA

BDMHDA was first examined for its toxicity against the gram-positive *B. megaterium* and gram-negative *E. coli*. The results showed that after the addition of 0.6 mM BDMHDA to LB cultures, both bacterial strains were inactivated within 10 min, suggesting a

bactericidal activity of BDMHDA. The bactericidal activity of BDMHDA was also tested by plating bacterial cell dilutions on LB-agar supplemented with different concentrations of BDMHDA. *E. coli* and *B. megaterium* did not survive even in the presence of 0.15 mM BDMHDA.

3.2. Removal of microorganisms by adsorption in batch experiments

The removal of bacteria by the micelle–montmorillonite complex was compared with that observed for loamy soil and montmorillonite (Table 1). Negligible removal was recorded for *E. coli* and *B. megaterium* by loamy soil and montmorillonite, whereas, the observed removal by ODTMA micelle-montmorillonite complex was six and seven logs for *E. coli* (Table 1). Similar removal efficiency was obtained for the bacteria with BDMHDA-micelle complex.

These results indicate that the removal of bacteria by the micelle–montmorillonite complex may depend on the surface composition of the bacterial cell.

Inhibition of bacterial growth could be detected in the supernatant when 10^7 and 10^4 CFU/ml of *A. brasilense* and *B. megaterium*, respectively, were treated with 0.2 g (in 20 ml) of BDMHDA–montmorillonite complex. A removal of 100% was recorded for both bacteria strains. Control experiments using quartz sand and quartz sand montmorillonite (without BDMHDA micelles) showed no significant reduction in bacteria concentration in both supernatants after treatment, suggesting that the BDMHDA–montmorillonite complex was responsible for the phenomenon of bacteria removal.

In order to assess the stability of bacteria–montmorillonite complex attachment, the BDMHDA–montmorillonite complex was removed by centrifugation from the supernatant, suspended in saline solution (0.9% NaCl) and vortexed at maximum velocity for 10 min. After sedimentation of the complex, the upper solution was plated on LB-agar and the bacteria were enumerated. No growth was detected for any treatment, suggesting that bacteria which were tightly attached to the complex were not released to the liquid medium or were dead (data not presented).

Table 1
Adsorption of representative bacteria to micelle–clay complexes

Microorganism	Adsorption matrix	Adsorption efficiency ($\log C_t/C_0$)[c]
E. coli K-12	Loamy soil	0.2 ± 0.37
	Montmorillonite	−0.1 ± 0.25
	ODTMA[a]	−7.1 ± 1.34
B. megaterium	BDMHDA[b]	−6.1 ± 0.13

[a]ODTMA.
[b]BDMHDA.
[c]C0, and Ct indicate the concentrations of microorganisms in suspension before and after treatment.

3.3. Effect of the micelle–montmorillonite complex on E. coli viability

Since it was not possible to test bacterial cell viability by a Biotium Inc. kit because the micelle–clay complex affected the observed fluorescence, we used the CO_2 resulting from bacterial respiration as indicator for *E. coli* viability. The effect of BDMHDA–montmorillonite complex on respiration activity of *E. coli* was measured by GC as CO_2 accumulation. The effect of the micelle–montmorillonite complex on *E. coli* viability was determined using different amounts of BDMHDA–montmorillonite complex and sand (Table 2). No respiration activity could be detected when *E. coli* cells were incubated with 900 mg BDMHDA-montmorillonite and 100 mg sand, or 500 mg BDMHDA-montmorillonite and 500 mg sand in comparison to the control, where *E. coli* cells were incubated with sand only and the respiration measured was significantly higher (Table 2). The results indicate that the micelle–montmorillonite complex possesses bactericidal properties which may result in bacterial death following such interaction i.e. *E. coli* cells from the suspension did not form colonies in LB solid medium.

The decrease of CO_2 levels below that in air in the presence of the positively charged micelle–montmorillonite complex (Table 2) is explained by the fact that CO_2

Table 2
Effect of BDMHDA–clay complex on respiration CO_2 accumulated in the vessel of *E. coli* after 24 h incubation (results represent three different experiments with three repeats each)

Vial	BDMHDA-CLAY (mg)	Sand	Bacteria	CO_2 (ppm)	SE**
1	100	900	10^7	193.2	±40.9c
2	300	700	10^7	204.5	±8.3c
3	400	600	10^7	215.9	±11.8c
4	500	500	10^7	244.3	±20.4c
6	0	1,000	10^7	3238.6	±170.4a
7	0	1,000	0	596.6*	±17b
Air	–	–	–	375	

*The respiration activity was derived apparently from microorganisms that are found in the non-sterile sand.
**Numbers (ppm CO_2) followed by the same letters were not statistically significant.

in contact with water is partly dissolved and yields the anion CO_3^-. This anion is partly adsorbed by the micelle–montmorillonite complex, which results in shifting the equilibrium towards further dissolution of CO_2.

3.4. Removal of microorganisms in filters of micelle–montmorillonite complex and sand

The results of the adsorption experiments showed that BDMHDA possesses apparent bactericidal activity as demonstrated by bacterial enumeration and respiration assays and that the BDMHDA micelle-montmorillonite system can remove selected microorganisms from contaminated water.

Evaluating the removal efficiency of microorganisms by filtration through columns packed with a mixture of the micelle–montmorillonite complex with excess sand (100:1; w/w) was compared with that of columns packed with sand and montmorillonite only (Table 3). Test microorganisms were seeded into 2–80 L of deionized water and pumped through the column at a flow rate of 20 ml/min. The concentration of microorganisms in the influent was 10^6 cfu/ml for *E. coli* and *B. megaterium*.

Reductions of 2.7 logs were recorded for *E. coli* in columns packed with sand–montmorillonite columns. Enhanced removal of the test microorganisms was accomplished using columns which included the micelle–montmorillonite complex (BDMHDA). Reductions of 4.73 and 4.91 logs were observed for *E. coli* and *B. megaterium*, respectively. In another filtration experiment with *E. coli*, a reduction by six orders of magnitude was observed (Table 3). This difference was within the estimated experimental error. We added for *E. coli* results of reduction for the passage of 30, 35, and 45 L (6, 4, and 1 Log units, respectively).

A more extensive study on *E. coli* removal by employment of two filters in series is presented in Table 4, in which average values of percent of bacterial removal of three separate experiments are presented. In this case, the volume passed was 80 L, and samples were taken from each column after every 5 L. These results were simulated by employing Eq. (1) for the analysis of filtration kinetics. The value of R_0, the molar concentration of binding sites of the bacteria in the filter, was determined from the number of bacteria adsorbed by the first filter, which was estimated to be 9×10^{10}, or 1.5×10^{-13} moles. Since the pore volume per column was 0.11 L, this amounts to $R_0 = 1.4 \times 10^{-12}$ M. In these calculations, we focused on simulating the results of filtration by the first column. The parameter C_1 was deduced from filtration of the first 20 L, since initially the adsorption sites are mostly unoccupied and the desorption of bacteria is expected to be a minor effect. The parameter D_1 was determined from the filtration results at the later stages.

The results of calculations Table 4 demonstrate a very good simulation of the filtration by the first column and a reasonable prediction for the number of bacteria emerging through the second column. A statistical analysis gave $R^2 = 0.964$. The value obtained for RMSE (Eq. (2)) was 2.3, which is well below the experimental error in most cases (Table 4).

To explore whether the bacteria inside the filter can be released and proliferated any more after their adsorption to BDMHDA, bacteria in samples from the center of the column were counted. It was not possible to detect *E. coli* colonies even without diluting the samples and plating on LB solid medium.

The results in Table 5 indicate 100% removal of *C. parvum* oocysts for the passage of 2 L, or 10^{10} oocysts per 1 kg of BDMHDA.

The micelle–montmorillonite complex and sand filters could be regenerated efficiently after treatment with 0.1% sodium hypochlorite and then washing with distilled water. The performance of the regenerated column filters in removing *E. coli* was essentially the same as that of first utilized columns.

Table 3
Reduction of *E. coli* and *B. megaterium* by filtration in columns packed with a mixture of a micelle–montmorillonite complex with excess sand (1:100, w/w)

Volume (L)	Microorganism	Log reduction in column packed with	
		Sand–montmorillonite column	Sand–montmorillonite complex (BDMHDA)
2	*E. coli*	−2.66 ± 0.8	−4.73 ± 1.8
2;30	*E. coli*		−6 ± 2
35	*E. coli*		−4 ± 2
45	*E. coli*		−1 ± 0.5
2	*B. megaterium*	ND[a]	−4.91 ± 0.6

[a]Not done.

Table 4
[a]Experimental and calculated percentage of removal of *E. coli* by two columns in series

Volume (L)	Column 1		Column 2	
	Experimental (%)	Calculated[b]	Experimental (%)	Calculated[b]
5	100 ± 0	99.8	100 ± 0	100
10	100 ± 0	99.7	100 ± 0	100
15	100 ± 0	99.5	100 ± 0	100
20	100 ± 0	99.2	100 ± 0	100
25	100 ± 0	98.8	100 ± 0	100
30	100 ± 0	98.2	100 ± 0	100
35	100 ± 0	97.4	100 ± 0	100
40	99 ± 0.3	96.2	100 ± 0	100
45	98 ± 0.6	94.6	100 ± 0	100
50	98 ± 0.8	92.3	100 ± 0	100
55	93 ± 4.2	89.2	100 ± 0	100
60	80 ± 10	85	100 ± 0	100
65	–	–	100 ± 0	100
70	70 ± 18.1	73	100 ± 0	100
75	61 ± 7.8	65.2	96 ± 0.9	99.9
80	53 ± 18.1	56.4	92 ± 4	99.8

[a]The number of *E. coli* bacteria in the water passing was 10^6 ml.
[b]The calculations according to Eq. (1) employed $R_0 = 1.45 \times 10^{-12}$ M; $C_1 = 9 \times 10^{11}\,M^{-1}\,min^{-1}$, and $D_1 = 2 \times 10^{-4}\,min^{-1}$.
The value of RMSE (Eq. (2)) was 2.3 and R^2 was 0.964.

Table 5
Removal of oocysts of *C. parvum* by the micelle–montmorillonite complex in filter experiments (1:100 w/w), Flow rate 20 mL

Cation type	*C. parvum* concentration in the Influent Oocyst/mL	Volume of treated water (L)	% Reduction of *C. parvum* by filtration (%)
BDMHDA	1×10^4	1	99.9
	1×10^4	2	99.4
	1×10^4	3	99.0
ODTMA	1×10^4	1	95.6
	1×10^4	2	87.4
	1×10^4	3	84.5
BDMHDA	1×10^4 [a]	1	100
	1×10^4 [a]	2	100

[a]In this case, the flow rate was 10 mL/min.

4. Discussion

We investigated the comparative removal from water of gram-negative *E. coli*, gram-positive *B. megaterium* and the protozoan *C. parvum* by micelle–montmorillonite complexes. We will first elaborate on the results of the extensive filtration experiment of *E. coli* in Table 4. The main purpose in the calculations in Table 4 was to demonstrate the availability of a procedure for estimating the capacity of a micelle–clay filter for purification of water from microorganisms under a variety of experimental situations. The calculations employed $D_1 = 2 \times 10^{-4}\,min^{-1}$, i.e. the dissociation of bacteria from the micelle–clay complex is rather slow, in accord with the results in suspension, or in filtration, which showed no desorption of bacteria from the complex. The value used for the forward rate constant of adsorption, $C_1 = 9 \times 10^{11}\,M^{-1}\,min^{-1}$, is very large; in fact, this value is 5-fold larger than $3 \times 10^9\,M^{-1}\,sec^{-1}$, which is the maximal possible value in the case of diffusion-controlled processes according to the Smoluchowski theory [22,23]. However, the interaction between a bacterium whose external surface is negatively charged and the micelle–clay complex involves an attractive force and is expected to involve more than one molecular attachment. The number of adsorbed bacteria which we used corresponds geometrically to a small fraction of the surface area of the micelle–clay complex in the filter.

The flow rate used of 20 mL/min corresponds to a flow velocity of 0.6 m/h. Calculations [24] indicate that for a longer filter, e.g. 2 m, a 10-fold larger flow rate, corresponding to a velocity of 6 m/h, would yield a somewhat larger capacity per kg of the complex then that obtained in the current study. Similarly, the calculations showed that the capacity per kg of the complex can be retained by using a higher flow rate and in proportion increasing the concentration of the active

ingredient. It should be emphasized that in the current experiments, the active component in the filter, the micelle–montmorillonite complex comprised just 1% of the weight of the filling mixture. We have recently developed a procedure for granulating the complex, which avoids the need to mix it with sand, thus yielding a 49-fold increase in the amount of complex per unit volume. The issue of filter capacity and economy for removal of microorganisms has to be further studied for chosen practical situations, but a filter including the micelle–montmorillonite complex can be employed right away in case of a sudden contamination of water by pathogenic microorganisms.

From the results (Table 4) the minimal value of bacteria which can be removed from synthetic water is 9×10^{10} per 2 g of the organic cation (BDMHDA or ODTMA), which is the only expensive component in the filter. This amounts to 4.5×10^{13} cfu/kg of organic cation. Jin et al. [25] presented the use of layered double hydroxides (LDH) for the removal of bacteria and viruses. They reported a removal from synthetic water of (1.4–2.1) 10^{10} pfu/kg LDH, and (3–5) 10^{13} cfu/kg LDH. When using raw river water, the removal efficiencies by LDH were 87–99%, but adsorption capacities were 4–5 orders of magnitude lower than in synthetic water. Recently, in the case of micelle–montmorillonite composites (Brook and Nir personal communication), tests of filtration of 15 L of gray water from showers and sinks, with a turbidity value of 3 NTU, total suspended solids (TSS) of 26 ppm and total bacterial count of 3×10^5 cfu/ml (which included about 30% of coliforms) yielded 99% removal and a total a removal of 4.5×10^9 cfu per 4 g of ODTMA, or 1.1×10^{12} cfu/kg ODTMA, whereas turbidity was reduced to 0.13 NTU and TSS to 1 ppm.

The results in Table 5 indicate that 10^{10} oocysts were removed per 1 kg of BDMHDA. For water contaminated with a relatively large number of *C. parvum* oocysts (1×10^3 oocysts/L), one kg of the cation might suffice the complete removal of *C. parvum* from 1,000 to 10,000 m^3, and a relatively large commercial filter, which includes 500 kg of the cation, might purify $5 \times 10^5 - 5 \times 10^6$ m^3 of highly contaminated water. Protozoa such as *C. parvum* resist removal by most disinfection techniques. In this study, at least four orders of magnitude removal were achieved by the micelle–montmorillonite complex. Thus, the system might possess a good potential for removing protozoa from contaminated waters.

An advantage of the micelle–montmorillonite complex in removal of bacteria from water, in comparison to other disinfection techniques, is that the microorganisms stay adsorbed by the complex within the column filter so that bacterial re-growth in the water is not likely. The bacteria seem to be both adsorbed by the complex and most likely inactivated, as shown by plating the filter material on LB nutrient-agar after the passage of bacteria, and by respiration experiments in suspension. Other species of gram-negative bacteria such as *Azospirillum brasilense* [26] and gram-positive bacteria such as *Bacillus subtilis*, streptococci from gray water and coliphages MS2 and fr were efficiently removed using micelle–clay complexes by the same procedures (unpublished results).

These results indicate that filters including micelle–montmorillonite composites that could be readily regenerated could secure the safety of drinking water for the population in case of threatening increase in the number of pathogenic microorganisms in the water.

5. Conclusions

Micelle–montmorillonite complexes of BDMHDA and ODTMA adsorbed efficiently bacteria such as *E. coli* and *B. megaterium*, and the parasite *C. parvum*. More than four-log reductions of *E. coli*, *B. megaterium*, and *C. parvum* were achieved in 20-cm-long columns of a micelle–montmorillonite complex mixed with excess sand (1:100, w/w). Bacterial cells adsorbed to micelle–montmorillonite complex lost their viability, indicating the bactericidal properties of the complex in addition to its adsorption capacity. The results of the column experiments demonstrated the suitability of the micelle–montmorillonite complex as a matrix for the purification of contaminated surface and groundwater from microbial and chemical pollutants; consequently, a cartridge of micelle–montmorillonite complex can be used as a point of use device for drinking water treatment.

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