Assessing the removal of inorganic colloids and Cryptosporidium parvum from drinking water

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A new batch device that simulates the conditions in water and wastewater treatment plants and enables the study of low-concentration feeds is described. The application of this apparatus to the monitoring of the concentration of inorganic and/or biological contaminants is demonstrated, using kaolin particles and Cryptosporidium parvum oocysts, respectively. The rate of inorganic particle attachment to the static medium is found to be directly proportional to the initial influent concentration. On the other hand, Cryptosporidium parvum removal is found to be more effective in the presence of additional (kaolin) particles, and this is attributed to electrostatic interactions between oppositely charged layers on the absorbing medium. Accordingly, the addition of humic materials is found to inhibit the removal process, possibly by neutralizing the positive charge on the kaolin particles. The relevance of these results to existing water purification processes in general and to risk assessment of microbiological contamination in particular is discussed.

Introduction

Maintaining and monitoring of water quality through the removal of chemicals and microorganisms is a key environmental issue.† Surface water contains various impurities of different sizes and properties, from semi-colloidal organic molecules (~0.1 μm) up to particles of 100 μm in diameter.‡ In this range, one also encounters pathogenic microorganisms, e.g. Cryptosporidium parvum and Giardia lamblia (the typical diameter of the infectious forms is 4–12 μm).§ Hence, surface water is not safe to drink, and requires prior physicochemical treatment.

Cryptosporidium parvum is a protozoan parasite that acts as a primary etiologic agent of enterocolitis in mammals.§ It causes cryptosporidiosis—an infectious gut disease. The protozoan oocyst is shed in high concentration (10⁷ oocysts mL⁻¹) in the feces of infected mammals and, unlike bacteria, C. parvum oocysts are biologically dormant and do not replicate outside their mammalian host.|| Human susceptibility to C. parvum is extremely high and as few as 10 oocysts can lead to infection.† The primary technique used for purifying drinking water is granular bed filtration.‡ This method is generally acknowledged to be robust and reliable for the removal of various contaminants.|| However, the treatment of C. parvum oocysts as if they were simple ‘dirt’ particles is oversimplified, as they cannot be removed efficiently in a manner similar to ordinary solid suspended impurities.‡

In order to study the dynamics of adhesion of particles of both inorganic and organic origins on the surface of quartz sand, we constructed an ideal-mixing batch reactor. One advantage of a batch reactor is the much simpler mass balance equation that includes only convection terms.‡

In this paper, we describe a specialized apparatus that simulates the conditions in water and sewage treatment plants. This device allows the continuous monitoring of the interactions of slurry particles with the filtering medium. The application of this equipment to monitor the removal of C. parvum oocysts and inorganic contaminants from water is discussed.

Experimental

Batch stirring reactor

Experiments were performed using a mechanical stirring apparatus composed of ten rubber-coated iron rods, each 25 cm in length and 4 cm in diameter (see Fig. 1). The rods were positioned in two rows, 13 cm apart, and attached with belts to an electrical motor, thus enabling horizontal rotation of the...
and 12 mg L⁻¹ mouse was orally inoculated with 10⁶ old immune compromised female C57/BL-6 mice, following a University of Arizona. The oocysts were propagated in 6 week Iowa strain oocysts obtained from Holstein bull calves at the preparation ensured dissolution and allowed a sufficient time for modification of the protocol reported by Yang et al. Samples were then purified using an 8.3 M (1.4 mg L⁻¹) solution, resulting in 99% purity. The final oocyst samples were dispensed into each sampling bottle to fill it up to the cork seals. In this method, particle detachment was monitored without the need to stop the rotation or to change the volume. Turbidity samples were collected every 8 min and analyzed immediately (Turbidimeter 2100P, Hach Co., Loveland, CO, USA). Sample turbidity was measured in nephelometric turbidity units (NTU). Samples for C. parvum enumeration were stored in a Biohazard refrigerator until analysis, which was performed within 72 h of sample collection. US EPA Method 1622, Cryptosporidium in Water by Filtration/Immunomagnetic separation (IMS)/Immunofluorescence assay (IFA) microscopy, was used. The sample was concentrated to a volume of 0.1 mL by means of centrifugation for 10 min at 3000 × g (Eppendorf 5403 centrifuge, Brinkmann Instruments, Westbury, NY, USA). Samples were stained with an indirect fluorescent monoclonal antibody (IFA). Cryptosporidium oocysts were enumerated using a Neubauer hemacytometer under a UV microscope at 40 × magnification (Carl Zeiss Microimaging, Thornwood, NY, USA).

Preparation of suspensions

Influent slurries were prepared from kaolin clay particles (Sigma-Aldrich Inc., St. Louis, MO, USA) and dechlorinated Cincinnati city tap water treated by reverse osmosis (RO). Approximately 870 mL of suspension, containing a predetermined concentration of kaolin particles of 0.78 ± 0.32 µm, was dispensed into each sampling bottle to fill it up to the cork seals to avoid the presence of air in the bottles. To ensure reproducibility, the slurry was mixed for 3 min using a regular kitchen blender (Betty Crocker 8 Speed Blender) at high speed. The bottles were sealed and laid down on the rods.

In experiments imitating the presence of natural organic matter (NOM) in surface water, a mixture of 10 mg L⁻¹ kaolin and 12 mg L⁻¹ sodium salt of humic acid (Sigma-Aldrich) in RO-purified water was used overnight under continuous stirring in a separate 10 L low-density polyethylene (LDPE) container. A suspension with a total organic carbon (TOC) concentration of 3.7 mg L⁻¹ in the filter influent was obtained. This preparation ensured dissolution and allowed a sufficient time for the sorption of organic material on the kaolin surface.

The stock solution of C. parvum oocysts was prepared from Iowa strain oocysts obtained from Holstein bull calves at the University of Arizona. The oocysts were propagated in 6 week old immune compromised female C57/BL-6 mice, following a modification of the protocol reported by Yang et al. Each mouse was orally inoculated with 10⁶ C. parvum oocysts in 200 µL of phosphate-buffered saline on the 8th day of the dexamethasone phosphate (0.288 mg L⁻¹) and tetracycline HCl (0.5 mg L⁻¹) regime, and transferred to suspension cages. Feces were collected every 36 h for the next 2–3 weeks. The samples were then purified using an 8.3 M (1.4 mg L⁻¹) CsCl solution, resulting in 99% purity. The final oocyst samples were suspended in phosphate-buffered saline solution (pH 7.4) containing antibiotics/antimicotic and stored at 4 °C until use in the experiments. An approximately 3 week to 1 month old titer of 10¹⁰ C. parvum oocysts was added to the slurry.

Preparation of flocculants

Alum [Al₂(SO₄)₃·18H₂O, purchased from Sigma-Aldrich] (20 mg L⁻¹), mixed with deionized water, was implemented as coagulant/flocculant for complete destabilization of the suspension. Cationic polyacrylamide C-475 (Cyanamid A/S, used as a coagulation agent) (0.2 mg L⁻¹) was added to achieve better adhesion.

Sampling procedure

Filtrate samples were collected using 10 mL disposable syringes with detachable needles that were inserted through vacuum seals. In this method, particle detachment was monitored without the need to stop the rotation or to change the volume. Turbidity samples were collected every 8 min and analyzed immediately (Turbidimeter 2100P, Hach Co., Loveland, CO, USA). Sample turbidity was measured in nephelometric turbidity units (NTU). Samples for C. parvum enumeration were stored in a Biohazard refrigerator until analysis, which was performed within 72 h of sample collection. US EPA Method 1622, Cryptosporidium in Water by Filtration/Immunomagnetic separation (IMS)/Immunofluorescence assay (IFA) microscopy, was used. The sample was concentrated to a volume of 0.1 mL by means of centrifugation for 10 min at 3000 × g (Eppendorf 5403 centrifuge, Brinkmann Instruments, Westbury, NY, USA). Samples were stained with an indirect fluorescent monoclonal antibody (IFA). Cryptosporidium oocysts were enumerated using a Neubauer hemacytometer under a UV microscope at 40 × magnification (Carl Zeiss Microimaging, Thornwood, NY, USA).

Batch experiments

The reactors were operated at a constant rotation speed of 0.5 rpm, and each experiment was carried out for 120 h. The kaolin concentration was changed from 10 to 900 mg L⁻¹. The tests were performed at room temperature (~20 °C). Immediately after each experiment, the sand was washed with deionized water, soaked in 0.1 M (2.4 g L⁻¹) HCl, dried at 110 °C and washed again with deionized water. To eliminate the risk of cross-contamination, sampling bottles were autoclaved, machine washed and heat dried before each use.

Results and discussion

Monitoring the removal of inorganic contaminants

In a typical experiment, a predetermined concentration of kaolin particles was slurried in water and charged to the batch reactor. The turbidity of the suspension was then monitored over 120 h. The influent concentration was changed from 10 to 900 mg L⁻¹ (see Fig. 2). In this set-up, however, it was impossible to limit the deposition of adsorbed particles. Thus, increasing the initial concentration of kaolin resulted in higher amounts adsorbed on the filter medium, up to 900(!) mg L⁻¹ (under flow conditions, a similar filter medium would be expected to accommodate a maximum of 100 mg L⁻¹ of impurities). This indicates that, under our batch reactor conditions, the hydrodynamic forces are suppressed. It is these forces that are responsible for the shearing of particle aggregates and, ultimately, for the dynamic equilibrium between the attachment and detachment processes (i.e. when the rates of the two processes are equal: \( k_{\text{attachment}} = k_{\text{detachment}} \)). Apparently, in our batch reactor, the kaolin particles insert between the filter granules, shifting the filter particles aside and increasing the overall volume of the filter bed. In the absence of hydrodynamic detachment forces, only electrostatic van der Waals’ and repulsion interactions remain. Another important finding was that \( k_{\text{attachment}} \) is directly proportional to the initial influent concentration. Thus, whilst...
it took 15 h to obtain a common treatment efficiency (CTE) of 0.7 log from the dilute suspensions [CTE defined as \( \log_{10}(C_{in}/C_{out}) \)], the same CTE was reached after only 10 h with the moderately concentrated samples. When concentrated suspensions were employed, a CTE of 0.7 log was reached after less than 2 h.\(^{23}\) We concluded that particle detachment forces are hydrodynamic forces, and that they may be suppressed under static bed conditions.

The above results can be explained by considering the elementary interactions between all the particles in the system. In the initial and final stages, the physical parameters of the absorbing medium are well defined. At first, the granules are devoid of any particles from the sludge (pristine medium) whilst, at the end of the ripening period, they are covered with an approximate monolayer of particles (mature medium). However, it is obvious that the transition between the two states is not a simple monotonous adsorption process, as this precludes any particle–particle interactions.\(^ {24,25}\) We propose that sludge particles that approach the pristine surface first adsorb to it and serve as a base for the construction of dendrites\(^ {26,27}\) – long particle chains which reflect particle–particle affinities. Once the dendrite chains have filled all the 'top' part of the absorbing bed, they flatten under the hydrodynamic forces and form an approximate monolayer covering the filter granules.

After this epitaxial layer has been formed, particles from the suspension will continue to attach to and detach from the coated granules. Although this is a dynamic process, the probability of attachment remains more or less constant, while the probability of detachment increases as a function of the number of adsorbed particles. Inevitably, dynamic equilibrium is reached (when \( k_{\text{attachment}} = k_{\text{detachment}} \)) which signifies the maturity of the absorbing bed.

Monitoring the removal of \( C. \text{parvum} \) oocysts

Here, we have observed two remarkable tendencies, which at first glance appear to be controversial with intuitive expectations: (i) it is more difficult to filter out \( C. \text{parvum} \) from ‘clean’ water (i.e. water which contains less colloid material); and (ii) as the filtration sequence progresses, more and more oocysts are filtered out. A series of blank experiments have confirmed that \( C. \text{parvum} \) oocysts do not adhere to the silica filter granules in the absence of kaolin or other clay particles.

The required presence of kaolin particles indicates that \( C. \text{parvum} \) adsorbs preferentially on these particles. It is known that both \( C. \text{parvum} \) oocysts and the filter medium are negatively charged. Therefore, the kaolin particles, which are structured as layers that are positively charged on their edges, could act as an electrostatic adhesive (see Fig. 3).

Besides particles of mineral origin, surface water contains dissolved contaminants such as humic acids.\(^ {28}\) These are negatively charged polyelectrolytes (mostly allomelanins from the decomposition of dead plants) that readily adsorb on clay surfaces.\(^ {29}\) The adsorption of humic acids has been shown to increase the negative surface charge and colloidal stability of clay colloids.\(^ {30}\) The humic material dyes the kaolin particles to a brown color, and effectively neutralizes the charge on the kaolin particles. Indeed, we found that, in the presence of

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**Fig. 2** Batch reactor kinetic experiments. Conditions: velocity, 0.5 rpm; 1 kg sand (\( d_{av} \sim 1.06 \text{ mm} \)); kaolin in tap water (10–900 mg L\(^{-1}\)). NTU.

**Fig. 3** Diagram showing how kaolin particles act as an electrostatic adhesive in the adsorption of \( C. \text{parvum} \) (CP) oocysts on sand granules.
humic acids, the efficacy of *C. parvum* removal was decreased by an order of magnitude.

Fig. 4 depicts the influence of organic material on turbidity (a) and *C. parvum* residual ratios (b). The residual ratio was compared 60 min into the ripening stage on filter medium with no flocculants, with alum alone and with a combination of alum and cationic polyelectrolyte. In the absence of humic materials, the residual ratio on a turbidity basis [Fig. 4(a)] increased from 0.3 log with no chemical additives, to 0.5 with alum, to 0.7 with alum and polyelectrolyte. The addition of humic material sharpened the changes in removal efficiency from 0.2 with no additives to 1.6 with a combination of chemical additives.

The removal efficiency of *C. parvum* [Fig. 4(b)] in the absence of humic materials increased from 1 log for the experiment performed with no addition of flocculants to 4.7 log with a combination of alum and polyelectrolyte. In the presence of humic materials, the oocyst removal efficiency decreased to 0.27 log with no flocculants and 1.52 log with a combination of alum and polyelectrolyte. This indicates that the neutralized, humic-coated kaolin particles have lost their adhesive properties.

Because we used humic acid in its sodium salt form, the overall ionic strength of the suspension was increased and the colloidal stability was reduced by depression of the double electric layer around the kaolin particles. At the same time, because the positively charged edges on the kaolin particles were coated, the specific adsorption of *C. parvum* oocysts on the kaolin surface was neutralized. These experiments confirm the assumption that kaolin functions as an additional retaining agent for *C. parvum* in filtration columns, and can also explain the previous findings of zero adsorption of *C. parvum* on bare sand without coagulants.

These findings can also account for the fluctuations observed for microorganism removal in water purification plants. When optimal chemical regimes were maintained throughout the whole year, the removal of oocysts was found to increase during periods of high water turbidity (i.e. when the water contained high amounts of clay particles). It follows that, rather than sticking to conventional and direct filtration schemes all year round, it is better to shift between methods (e.g. use contact filtration during the summer months, when the water turbidity is usually lower).

**Conclusions**

The continuous monitoring of the removal of *C. parvum* oocysts and inorganic contaminants from water can be achieved using a new ideal-mixing batch reactor. The poor adsorption of *C. parvum* on sand particles may be attributed to electrochemical repulsion, as suggested by experiments in the presence of ‘filler’ compounds, such as kaolin particles, and by the formation of negatively charged layers in the presence of both kaolin and humic materials. Removal of inorganic particles, the major contributors to water turbidity, may have a negative effect on the removal of *C. parvum* oocysts. Thus, the implementation of a conventional filtration scheme may cause sufficient reduction of turbidity prior to filtration, and such low values of water turbidity may lead to the misinterpretation of microbiological contamination risks.

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**References**

15 In comparison, the usual plug flow reactor (PFR, the so-called filtration column) is a much more complicated device in terms of mass balance, as both diffusion and convection terms must be included in the mass balance equation.


19 Control experiments showed that simple mechanical stirring of the sand particles gave irreproducible results, due to particle grinding and breakdown which resulted in increased turbidity. Using our ‘external stirring’ method, the error due to this was less than 1%.


22 Numerous systems are available for the detection of oocysts in water supplies. Although the number of detected oocysts in the method used here (IFA) is the exact number that correlates with other known methods of physical detection of the parasite (e.g. turbidity and particle counting), this method does not provide information regarding the viability or infectivity of the samples. Several viability assays, including excystation assays and vital dye staining, have been used to predict the viability of *C. parvum* oocysts found in water (see C. R. Fricker and J. H. Crabb, *Adv. Parasitol.*, 1998, 40, 241). However, these methods may overestimate the number of infective oocysts present in environmental samples (cf. S. C. Weir, N. J. Pokorny, R. A. Carreno, J. T. Trevors and H. Lee, *J. Parasitol.*, 2001, 87, 1502). Infectivity of *C. parvum* oocysts is determined either by *in vivo* infectivity of animal hosts or in a cell culture assay. Although animal infectivity assays are useful for the detection of infectious *C. parvum* oocysts in water, cell culture allows for quantitative measurements (T. R. Slifko, D. E. Huffman and J. B. Rose, *Appl. Environ. Microbiol.*, 1999, 65, 3936). This method involves infection of a cultured cell line with *C. parvum* oocysts and subsequent detection with a fluorescent antibody. Foci of infection are viewed by epifluorescent microscopy. This method is called the focus detection method (FDM).

23 Although different particle removal values were observed for the various suspensions, the normalized removal fractions \(1 - C/C_0\) remained between 0.8 and 0.9 throughout.


33 Optimal chemical regime refers to the addition of predetermined amounts of multivalent ions to decrease the surface charge on the particle. For particles of certain origin, the type and concentration of these ions can have a crucial effect on the attachment of the particle to the grains of the medium.