



Comparative reductions of *Cryptosporidium parvum* oocysts, *Bacillus subtilis* spores, uncoated and glycoprotein-coated microspheres during water filtration through quartz sand

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ARTICLE INFO

Editor: Li Gao

Keywords:

Cryptosporidium parvum
Surrogate
Microspheres
Spores
Reduction
Filtration

ABSTRACT

Contamination of drinking water by *Cryptosporidium parvum* oocysts poses a significant public health risk, as evidenced by numerous outbreaks of cryptosporidiosis worldwide. This study evaluated the effectiveness of *Bacillus subtilis* spores, unmodified yellow-green (YG) and yellow-orange (YO) microspheres, and glycoprotein-coated YO microspheres, in predicting *C. parvum* oocyst reduction during water filtration through quartz sand. Column filtration experiments were conducted at a flow rate relevant to slow sand filtration using Vienna tap water. Concentration breakthrough curves and data analysis using colloid filtration theory revealed the reduction order as *B. subtilis* spores \ll YG microspheres \ll glycoprotein-coated YO microspheres $<$ *C. parvum* oocysts $<$ YO microspheres. The normalized concentrations (C/C_0) were in the range of 10^{-2} for spores, 10^{-3} for YG microspheres, 10^{-4} for both glycoprotein-coated YO microspheres and oocysts, and 10^{-5} for YO microspheres. Under the experimental conditions of this study, *B. subtilis* spores and YG microspheres were overly-conservative surrogates, while YO microspheres were under-conservative surrogates. Comparatively, glycoprotein-coated microspheres provided the closest predictions in oocyst reduction, though slightly conservative. The differences or similarities in physicochemical properties (size, shape, surface charge, hydrophobicity) and surface macromolecules between the oocysts and candidate surrogates were considered to be the determining factors influencing surrogate effectiveness. Glycoprotein-coated microspheres, exhibiting similar physicochemical properties to oocysts, emerged as the most effective surrogate, providing an accurate, albeit slightly conservative, prediction of oocyst reduction in sand media. The study highlights the importance of selecting appropriate surrogates for effective water treatment design and operation, balancing safety margins and cost efficiency.

1. Introduction

Contamination of drinking water by *Cryptosporidium parvum* protozoa poses a significant public health risk. *C. parvum* oocysts are frequently detected in source waters for drinking water supplies. Insufficient removal of these resilient parasites during water treatment has caused numerous cryptosporidiosis outbreaks worldwide [1–5],

including the 1993 Milwaukee outbreak affecting >400,000 people [6]. These events underscore the critical need for effective water treatment strategies to ensure the safety of drinking water supplies and prevent future outbreaks.

Surrogates are valuable tools for assessing the effectiveness of water treatment processes and informing treatment design. Many researchers have employed bacterial spores [7,8] and unmodified oocyst-sized

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Table 1
Zeta potential of colloids measured in Vienna tap water and 10 mM NaCl.

	Zeta potential (mV)	Standard deviation (mV)
In Vienna tap water (electrical conductivity 273 $\mu\text{S}/\text{cm}$, pH 7.8), related to the column experiments:		
<i>Cryptosporidium parvum</i> (irradiated)	-12.48	3.74
<i>Cryptosporidium parvum</i> (active)	-13.10	4.64
YG microspheres	-41.69	1.42
YO microspheres	-4.89	0.91
Glycoprotein-coated YO microspheres	-9.15	1.59
<i>Bacillus subtilis</i> spores	-18.40	1.25
In 10 mM NaCl (electrical conductivity 1044 $\mu\text{S}/\text{cm}$, pH 8.0):		
<i>Cryptosporidium parvum</i> (irradiated)	-10.28	3.47
<i>Cryptosporidium parvum</i> (active)	-19.67	8.82
YG microspheres	-47.57	1.36
YO microspheres	-6.52	1.08
Glycoprotein-coated YO microspheres	-10.24	1.35
<i>Bacillus subtilis</i> spores	-30.85	0.64

Abbreviation: YG - yellow-green, YO - yellow-orange.

NaCl.

2.2. Sample enumeration

The *C. parvum* oocysts were quantified using solid-phase cytometry (ChemScan RDI; AES Chemunex, bioMérieux, Marcy l'Étoile, France), following the method established in Stevenson et al. [15], where a calibration curve using this new method, is presented in the supplemental information of that publication. In short, each ChemScan filter, onto which an oocyst sample was filtered, was placed on 20 μl of *EasyStain*TM (BTF Pty. Ltd., bioMérieux) and 30 μl of sterile deionized water, on a petri dish, and incubated for 15 min at 37 °C. After allowing the filters to dry, 150 μl of fixing buffer (bioMérieux) was applied to each filter. The filters were then allowed to incubate at room temperature for 2 min, before laying the filter on absorbent paper to dry. Once the filter was dry, it was placed on a support pad that was saturated with 100 μl phosphate buffer solution on a ChemScan holder. In this way, the oocysts could then be enumerated using solid-phase cytometry, along with the microspheres which were also filtered onto ChemScan filters, as outlined in Stevenson et al. [23]. Specific discrimination settings were used for the oocysts, which had weaker fluorescence relative to the microspheres. The ChemScan RDI can identify microspheres and microorganisms in three color wavelength ranges: green, orange, and dark red. The *EasyStain* and YG microspheres have an excitation maximum of 441 nm and an emission maximum of 486 nm, while the YO microspheres have an excitation maximum of 529 nm and an emission maximum of 546 nm. Three replicates were analyzed for each data point and the average was calculated. For determining the influent solution concentrations, samples were taken at the beginning and at the end of the injection time, in order to confirm that the influent concentration remained stable throughout the experiments. The influent concentration was calculated as an average of these values.

B. subtilis spores were enumerated by the plaque counting method as described in Oudega et al. [42]. This involved heat treating the samples at 70 °C for 10 min in order to inactivate the vegetative cells, within 24 h of sampling. Subsequently, the samples were incubated at 36 \pm 2 °C for 44 \pm 4 h on plate count agar (Tryptone Glucose Yeast Extract, Oxoid Ltd., Hampshire, U.K.), after which the plaques could be counted. The conservative tracer, NaBr, breakthrough was detected using a flow-through cell and a portable electrical conductivity meter (WTW Tetra-Con 325 and ProfiLine Cond 3310, Xylem Analytics, Weilheim, Germany).

2.3. Column filtration experiments

Colloid filtration experiments were performed using columns made of 30 cm long Plexiglas tubes, with an inner diameter of 7 cm. The columns contained laboratory-grade coarse quartz sand with a grain size of 0.4–0.8 mm (Carl Roth, Germany) and a bulk density of 2.65 g/cm³. The packed sand media were fully saturated with Vienna tap water. Vienna tap water is sourced from groundwater, and therefore, it was chosen as a representative groundwater matrix for the column filtration experiments. The saturated porosity was determined volumetrically to be 0.43. The experiments were conducted at a flow rate 19 \pm 0.89 ml/min, pumped upward using a peristaltic pump (Masterflex, Vernon Hills, IL), and the outflow samples were collected continuously in 15 ml test tubes throughout the experiments. At least 20 pore volumes (one pore volume \sim 500 ml) of Vienna tap water were pumped through the column before each experiment to equilibrate the column.

Prior to the experiments, colloidal solutions were homogenized by submersing in an ultrasound bath for 3 min to minimize aggregation. Careful attention was paid to keeping similar injection concentrations, so as not to produce an effect from influent concentration [24]. The oocyst and microsphere suspensions were injected at a concentration of 1 \times 10⁴–5 \times 10⁴ particles/mL while the *B. subtilis* spores were injected at 4 \times 10⁶ particles/mL, due to the limit of detection. In all the experiments, two pore volumes (\sim 1 l) of each colloid solution was injected, followed by flushing with tracer-free Vienna tap water. A conservative, non-reactive solute tracer, NaBr (1 mM), was co-injected with all colloidal tracers to indicate the flow rate. An exception was in the oocyst experiments; NaBr was not used in order to minimize contamination of the measuring equipment. Experiments were performed in duplicate except for the experiments with *B. subtilis* and glycoprotein-coated microspheres due to limited stocks.

2.4. Data analysis

Peak breakthrough attenuation was estimated from the log₁₀ reduction value (*LRV*) of the input concentration (*C*₀) over the peak outflow concentration (*C*_{max}):

$$LRV = \text{Log}_{10} \frac{C_0}{C_{\text{max}}} \quad (1)$$

The peak breakthrough attenuation marks the point at which the highest concentration of the particles breaks through the porous media and appears in the outflow. Therefore, *LRV* represents the most critical scenario for assessing the efficiency of the filtration medium. Relative mass recovery (*RB*) was estimated by integrating the area under the breakthrough curve and normalizing to the conservative tracer breakthrough (bromide):

$$RB (\%) = \frac{\left(\int \frac{C}{C_0} dt \right)_{\text{Colloids}}}{\left(\int \frac{C}{C_0} dt \right)_{\text{NaBr}}} \times 100\% \quad (2)$$

in which, *C*/*C*₀ is the outflow concentration (*C*) relative to the input concentration (*C*₀), and *t* is the time.

To derive pore-water velocity (*V*) and the hydrodynamic dispersion coefficient (*D* = λV , λ is the hydrodynamic dispersivity), experimental data of the NaBr tracer were simulated using the 1-D advection-dispersion transport model encoded in STANMOD (version 2.07):

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - V \frac{\partial C}{\partial x} \quad (3)$$

in which, *x* is the transport distance. Colloid filtration theory was used to derive sticking efficiency (α) and the deposition rate constant (*K*) of particles, as follows [25]:

$$\alpha = \frac{d \left[\left(1 - 2 \frac{\ln(RB)}{x} \right)^2 - 1 \right]}{6(1-\theta)\eta_0 \lambda} \quad (4)$$

$$K = \frac{3(1-\theta)Ua\eta_0}{2d\theta} \quad (5)$$

where d is the effective grain size of the porous media, θ is the effective porosity, η_0 is the single-collector contact efficiency [26], and U is the Darcy velocity ($U=V\theta$). The η_0 , α and K values were calculated using the equations given in Tufenkji and Elimelech [26]. Table 2 lists the specific parameter values used in the calculations.

3. Results and discussion

3.1. Relative reduction

NaBr breakthrough curves (BTCs) derived from individual experiments are very similar (Fig. 2) indicating consistent flow conditions among the column experiments, which allows a direct comparison of the various colloids for their reduction and transport. An exception is the NaBr BTC for the experiment with the spores, which appeared earlier, suggesting a faster flow rate. This is further confirmed by the results of STANMOD modelling (Table 3). Pore-water velocity optimized from the model is 13–15 m/day (0.83–1.03 cm/min), which is a Darcy velocity of 5.59–6.45 m/day, consistent with typical flow rates observed in slow sand filtration used in water treatment [27,28].

The colloid BTCs (Fig. 3) indicate that *B. subtilis* spores and YG microspheres significantly over-predicted oocyst relative concentrations, with C_{\max}/C_0 values 264 and 29 times higher, respectively. Spores and YG microspheres underestimated oocyst LRV by 2.4 and 1.5 log respectively, and overestimated oocyst mass recovery by 207 and 42 times, respectively (Table 4). In comparison, glycoprotein-coated YO microspheres somewhat overestimated oocyst C_{\max}/C_0 and mass recovery by 3.0 and 2.9 times, respectively, while underestimating LRV by 0.5 log. YO microspheres, on the other hand, slightly underestimated oocyst C_{\max}/C_0 and mass recovery by 1.4 and 2.1 times, respectively,

Table 2
Specific parameter values used in the colloid filtration theory calculations.

Parameter	Units	Value	Reference
Effective grain size of sand	mm	0.40	This study
Temperature	Kelvin	295.15	22 °C
Fluid viscosity at 22 °C	kg/(m.s)	9.55×10^{-4}	
Particle diameter:			
<i>Bacillus subtilis</i> spores	µm	0.5×1.5	Measured this study
<i>C. parvum</i> oocysts	µm	4.86	[13]
Glycoprotein-coated YO microspheres	µm	4.95	[13]
Uncoated microspheres	µm	4.50	Polysciences Inc
Particle buoyant density:			
<i>Bacillus subtilis</i> spores	kg/m ³	1335	[35]
<i>C. parvum</i> oocysts	kg/m ³	1050	[19]
Polystyrene microspheres	kg/m ³	1050	Polysciences Inc
Hamaker constant:			
Oocysts-water-quartz	J	6.50×10^{-21}	[40]
<i>Bacillus subtilis</i> spores	J	6.50×10^{-21}	[40]
Polystyrene -water-quartz	J	6.79×10^{-21}	[41]

Note: YO: yellow-orange.

while overestimating LRV by 0.3 log.

BTCs and data analysis results (Table 4) indicate that the degree of colloid reduction is in the order of *B. subtilis* spores \ll YG microspheres \ll glycoprotein-coated YO microspheres $<$ *C. parvum* oocysts $<$ YO microspheres. This is reflected in the decreasing values of C_{\max}/C_0 and RB , and increasing values of LRV, α , η and K (Table 4). Fig. 3 demonstrated that the normalized concentrations (C/C_0) were approximately 10^{-2} for spores, 10^{-3} for YG microspheres, 10^{-4} for both glycoprotein-coated YO microspheres and oocysts, and 10^{-5} for YO microspheres.

The above results demonstrate that glycoprotein-coated oocyst-sized microspheres outperform both *B. subtilis* spores and unmodified microspheres in predicting oocyst reduction in quartz sand. This superiority over unmodified microspheres aligns with findings from previous studies in alluvial sand [13], limestone sand [15], filter sand [14], and sand-anthracite dual-media [16]. The factors and mechanisms contributing to the differences described above are discussed below.

3.2. *B. subtilis* spores

One reason for the significant difference in reduction between *B. subtilis* spores and *C. parvum* oocysts is their size disparity, with oocysts being nearly 6 times larger in diameter. The size ratio of spores to quartz sand grains (0.21 %) is below the 0.7 % threshold value for straining to become an important factor affecting colloid retention [29]. In contrast, the larger size ratio of oocysts to sand grains (1.22 %) exceeds this threshold, making straining a major factor in their removal. This difference in size likely contributes to the lower removal of spores compared to oocysts. Additionally, the shape difference between rod-shaped spores and spherical oocysts can also influence their deposition and transport through porous media, possibly due to tumbling and alignment with flow paths, as was shown with differently shaped microplastics [30].

Compared to the oocysts, *B. subtilis* spores are more negatively charged (Table 1) in both Vienna tap water (−18.40 mV vs. −12.48 mV) and 10 mM NaCl (−30.85 mV vs −10.28 mV). The greater negative charge could also have contributed to the lower reduction of the spores due to a greater repulsion from the sand grain surfaces. The zeta potential values measured in this study are similar to the inactivated oocysts (−12.8 mV in 1 mM NaCl pH 7; [13]), and spores (−30 mV; [31]) in pH 7.5 (unspecified ionic strength). Similarly, Liu et al. [14] reported a zeta potential of −10.9 mV for viable oocysts in 1 mM NaCl.

The shapes of BTCs of the spores and oocysts are very different despite their same injection volume (Fig. 3). The spore BTC shows a gradual rising limb, a very narrow peak, and a very sharp falling limb, with long tailing. The gradual rising limb of the spore BTC suggests that spores undergo reversible attachment and detachment processes. They attach to surfaces and then detach over time, causing a slower initial increase in concentration. The long tailing also indicates detachment because of the reversible attachment. Due to their smaller particle size, the likelihood of reversible attachment, and a lack of straining, the spores are readily dislodged from the previously attached surfaces during their transport, resulting in less reduction than oocysts. In contrast, BTCs of oocysts show sharp rising and falling limbs with a plateau and little tailing. Due to their larger size, oocysts would have more limited access to smaller pores. This can lead to a faster initial breakthrough as a significant portion of the oocysts may bypass smaller pores and travel more directly through larger pore networks. A lack of long tailing means that once the oocysts attach to surfaces, they tend to stay attached, indicating a more irreversible attachment process.

B. subtilis spores and *C. parvum* oocysts have very different hydrophobicity characteristics. *C. parvum* oocysts are highly hydrophilic [14]. In contrast, *B. subtilis* spores are highly hydrophobic as they have a strong affinity for hexadecane and other hydrophobic solvents [32]. Based on tests of bacterial adherence to hexadecane, relative hydrophobicity is 92–95 % for *B. subtilis* spores [33] but only 16.8 % \pm 1.8 % for inactivated *C. parvum* oocysts [34]. Based on contact angle

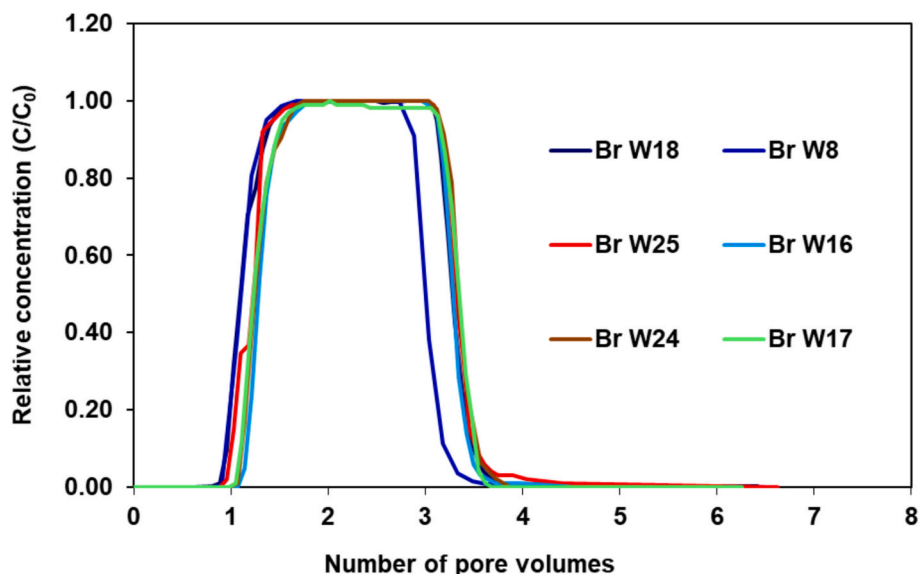


Fig. 2. Relative NaBr concentrations observed during the column filtration experiments.

Table 3

Flow parameter values derived from NaBr data using the STANMOD model.

Colloid	Test	Pore-water velocity V (cm/min)	Dispersion coefficient D (cm ² /min)	Pulse duration (min)	r^2
<i>Bacillus subtilis</i> spores	W8	1.03	0.17	50	1.00
YG microspheres	W16	0.88	0.10	53	1.00
YO microspheres	W17	0.83	0.14	60	1.00
Glycoprotein-coated YO microspheres	W18	0.99	0.24	59	1.00
YO microspheres	W24	0.90	0.14	55	1.00
YG microspheres	W25	0.92	0.19	58	0.99

Note: YG: yellow-green, YO: yellow-orange.

measurements, Liu et al. [14] derived a hydrophobicity of 8.4 % for *C. parvum* oocysts. The hydrophobic nature of the spores suggests that in the presence of hydrophobic materials, such as organic matter, hydrophobic interactions can play an important role in spore adhesion to surfaces by enhancing their attachment; however, a minimal influence of hydrophobic interactions on spore attachment to the quartz sand investigated in this study is expected, due to a lack of organic carbon. Additionally, the buoyant density of *B. subtilis* spores (1.335 g/cm³, [35]) is greater than that of *C. parvum* oocysts (1.05 g/cm³, [19]), although, the effect of buoyant density on their reduction is considered to be minor.

3.3. Unmodified microspheres

Despite having the same particle size (4.5 μm), YG and YO microspheres exhibited significantly different reduction characteristics. YG microspheres significantly under-predicted oocyst reduction, whereas YO microspheres slightly over-predicted oocyst reduction. Their different zeta potentials, resulting from the presence of different fluorescent molecules, are likely a major contributing factor to these contrasting reduction behaviours. YG microspheres were significantly more negatively charged than the oocysts (zeta potential −41.69 mV, in Vienna tap water), whereas YO microspheres were less negatively charged than the oocysts (zeta potential −4.89 mV, in Vienna tap water).

Similar to oocysts, straining is likely a significant factor influencing microsphere retention, as the size ratio of the microspheres to sand

grains was 1.13 %, exceeding the 0.7 % threshold value. Liu et al. [14] reported that unmodified microspheres are more hydrophobic than oocysts (relative hydrophobicity 27.2 % vs. 8.4 %, based on contact angle measurements). However, as discussed in Section 3.2, hydrophobic interactions are considered insignificant under the experimental conditions investigated in this study. Both YG and YO microspheres have similar sizes and buoyant densities to the oocysts (Table 3). However, unlike the oocysts, unmodified microspheres lack surface macromolecules.

The BTCs of YG microspheres consistently exhibited double peaks. The BTCs of the first peaks (1.2–3.3 PVs) exhibited similarities to the BTCs of oocysts, displaying sharp rising and falling limbs with a plateau. The second peaks were retarded (3–5 PVs in experiment W16 and 4–9 PVs in experiment W25) and exhibited tailing. The double-peaked BTCs of YG microspheres likely reflect the presence of multiple transport pathways and mechanisms within the quartz sand, with the first peak representing a faster, less retained population of microspheres, and the second peak representing a population experiencing more complex interactions and potentially retardation and reversible attachment.

The BTCs of YO microspheres exhibited a markedly different pattern from those of the oocysts and YG microspheres. The BTCs displayed significant irregularity without a plateau and were not well-defined, with more scatter, likely attributed to enumeration of low concentrations.

3.4. Glycoprotein-coated microspheres

Similar to the BTCs of oocysts, the BTC of the glycoprotein-coated microspheres displays sharp rising and falling limbs, but instead of a plateau, it exhibits double peaks (0.6–1.6 and 1.8–2.4 PV). The double peaks in the modified microsphere BTC might be due to heterogeneous attachment and different transport pathways. The modified microspheres may attach to the sand medium in different ways, with some attaching more strongly and releasing more slowly than others. This could lead to multiple phases of release. The modified microspheres may also follow different pathways through the sand media, with some taking longer routes than others. This could also lead to multiple peaks in the BTC. With a similar particle size to oocysts and a size to sand grain ratio (1.24 %) > 0.7 %, straining is also considered important in their attenuation and transport.

Glycoprotein coating effectively shifted the zeta potential of YO microspheres from −4.89 mV to −9.15 mV, bringing them closer to that

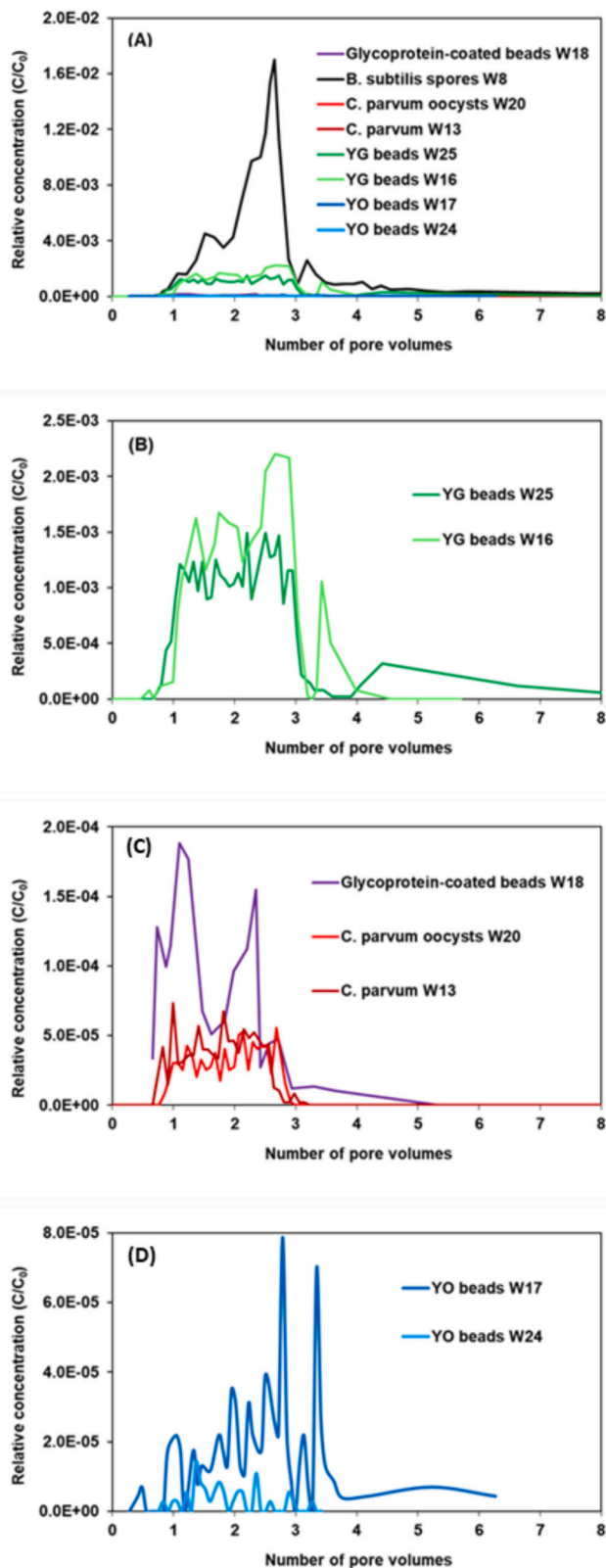


Fig. 3. Relative concentrations of *C. parvum* oocysts, *B. subtilis* spores, unmodified YG and YO microspheres, and glycoprotein-coated YO microspheres observed during the column filtration experiments. Note the difference in y-axis scales: (A) 10^{-2} , (B) 10^{-3} , (C) 10^{-4} , and (D) 10^{-5} .

of oocysts (-12.48 mV). This is attributed to the similar isoelectric points of the glycoprotein and oocysts ($\text{pH} \approx 2$, [13]). Glycoprotein coating also mimics the surface macromolecules of oocysts, as glycoproteins are the predominant proteins on their surfaces and form a glycoprotein outer layer on oocyst walls [36–38]. It has been found that surface macromolecules play a significant role in the attachment of *C. parvum* oocysts to solid surfaces [37,39].

Furthermore, it can be expected that the glycoprotein coating would have also reduced the hydrophobicity of YO microspheres. For example, glycoprotein coating of $4.5 \mu\text{m}$ bright blue microspheres shifted the relative hydrophobicity from 74 % to 26 %, bringing it closer to that of viable oocysts (17 %), based on hexadecane adherence tests (authors' data). Liu et al. [14] have also demonstrated that glycoprotein-coated microspheres were less hydrophobic than unmodified microspheres. Additionally, it is expected that the modified microspheres have a very similar buoyant density to the oocysts (1.05 g/cm^3), as well as the unmodified microspheres. However, the effects of hydrophobicity and buoyant density are minor under the experimental conditions investigated in this study.

4. Conclusions

Our experimental results demonstrate that, compared to *B. subtilis* spores and unmodified oocyst-sized microspheres, glycoprotein-coated microspheres are a better surrogate for predicting *C. parvum* oocyst reduction in quartz sand media. This is attributed to several similar physicochemical properties (size, shape, zeta potential, hydrophobicity, buoyant density) and surface glycoprotein macromolecules. As the removal of YO biomolecule-modified and YO unmodified microspheres exhibited similar transport to the oocysts, we can conclude that size and surface charge are the two dominant properties determining the transport and removal of *C. parvum* in coarse quartz sand media. Under the experimental conditions of this study, glycoprotein-coated microspheres were a somewhat conservative surrogate for predicting oocyst reduction. This is advantageous because a conservative, yet not overly conservative, surrogate provides a safe margin in its predictions.

Findings from this study indicate that *B. subtilis* spores are not an accurate surrogate for predicting oocyst reduction in quartz sand, being overly conservative, as they significantly underestimated oocyst reduction. This discrepancy can be explained by the distinct physicochemical properties of spores and oocysts. Unmodified oocyst-sized YG microspheres also significantly underestimated oocyst reduction, acting as another overly conservative surrogate. Conversely, YO microspheres slightly overestimated oocyst reduction, serving as an under-conservative surrogate. These differences can be attributed to the contrasting zeta potentials of the microspheres, resulting from different fluorescent dyes.

Accurate surrogate selection is crucial for effective water treatment design and operation. Overly conservative surrogates can lead to over-engineered systems, increased costs, and unnecessary public concern. Conversely, under-conservative surrogates can underestimate risks, compromising water quality and public health. Therefore, it is essential to identify and use surrogates that have multiple physicochemical property similarities and closely mimic the behaviour of the target pathogens. Bio-molecule modified surrogates offer an alternative to standard practices. Future work should evaluate the reduction of *B. subtilis* spores in comparison to unmodified and glycoprotein-coated microspheres in various natural aquifer materials.

CRedit authorship contribution statement

M.E. Stevenson: Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **L. Pang:** Writing – original draft, Formal analysis. **A.H. Farnleitner:** Writing – review & editing, Conceptualization. **G. Lindner:** Investigation. **A.K.T. Kirschner:**

Table 4

Colloid reduction during water filtration through quartz sand, sorted ascendingly.

Colloid	Test	From breakthrough curves			From colloid filtration theory		
		C_{\max}/C_0	LRV	RB (%)	α	η	K (min^{-1})
<i>Bacillus subtilis</i> spores	W8	1.70×10^{-2}	1.77	8.09×10^{-1}	0.05	3.30×10^{-4}	0.01
YG microspheres	W16	2.20×10^{-3}	2.66	1.77×10^{-1}	0.30	2.71×10^{-3}	0.05
YG microspheres	W25	1.49×10^{-3}	2.83	1.47×10^{-1}	0.35	3.03×10^{-3}	0.06
	Average	1.85×10^{-3}	2.74	1.62×10^{-1}	0.33	2.87×10^{-3}	0.06
Glycoprotein-coated YO microspheres	W18	1.88×10^{-4}	3.73	1.13×10^{-2}	0.77	7.25×10^{-3}	0.15
<i>Cryptosporidium parvum</i>	W13	7.35×10^{-5}	4.13	4.56×10^{-3}	0.87	8.57×10^{-3}	0.16
<i>Cryptosporidium parvum</i>	W20	5.55×10^{-5}	4.26	3.26×10^{-3}	0.93	9.12×10^{-3}	0.17
	Average	6.45×10^{-5}	4.19	3.91×10^{-3}	0.90	8.85×10^{-3}	0.17
YO microspheres	W17	7.88×10^{-5}	4.10	3.42×10^{-3}	0.99	9.14×10^{-3}	0.16
YO microspheres	W24	1.42×10^{-5}	4.85	3.79×10^{-4}	1.46	1.28×10^{-2}	0.25
	Average	4.65×10^{-5}	4.48	1.90×10^{-3}	1.22	1.10×10^{-2}	0.20

YG: yellow-green; YO: yellow-orange; C_{\max} : peak concentration; C_0 : injection concentration; LRV: \log_{10} reduction value, RB: mass recovery relative to NaBr; α : sticking efficiency; η : single-collector removal efficiency; K: deposition rate constant.

Writing – review & editing, Resources. **A.P. Blaschke**: Conceptualization. **R. Sommer**: Writing – review & editing, Resources, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded in whole or in part by the Austrian Science Fund (FWF) T970-N29. For open access purposes, the author has applied a CC BY public copyright license to any author accepted manuscript version arising from this submission. This is a joint research effort of the Interuniversity Cooperation Centre for Water & Health (www.waterandhealth.at).

Data availability

Data will be made available on request.

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