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Review

A new workflow for assigning removal credits to assess overall performance of managed aquifer recharge (MAR)

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ABSTRACT

Pathogen removal in managed aquifer recharge (MAR) systems is dependent upon numerous operational, physicochemical water quality, and biological parameters. Due to the site-specific conditions affecting these parameters, guidelines for specifying pathogen removal have historically taken rather precautionary and conservative approaches in order to protect groundwater quality and public health. A literature review of regulated pathogens in MAR applications was conducted and compared to up-and-coming indicators and surrogates for pathogen assessment, all of which can be gathered into a toolbox from which regulators and operators alike can select appropriate pathogens for monitoring and optimization of MAR practices. Combined with improved knowledge of pathogen fate and transport obtained through lab- and pilot-scale studies and supported by modeling, this foundation can be used to select appropriate, site-specific pathogens for regarding a more efficient pathogen retention, ultimately protecting public health and reducing costs. This paper outlines a new 10 step-wise workflow for moving towards determining robust removal credits for pathogens based on risk management principles. This approach is tailored to local conditions while reducing overly conservative regulatory restrictions or insufficient safety contingencies. The workflow is intended to help enable the full potential of MAR as more planned water reuse systems are implemented in the coming years.

1. Introduction

Managed aquifer recharge (MAR) systems have been used for nearly a century to intentionally recharge groundwater using surface water, stormwater, or reclaimed water (secondary or tertiary treated effluents or advanced treated water (National Research Council, 2008)) in multiple applications throughout the world. Typically, water can either be applied via surface spreading basins during soil aquifer treatment (SAT), directly recharged into the subsurface using either dry wells or infiltration galleries via the vadose zone, or be injected into the saturated zone of an aquifer via aquifer storage and recovery (ASR). While SAT and associated rapid infiltration basins (RIBs) can receive surface water, stormwater, and reclaimed water, ASR applications require water with a higher degree of pretreatment, usually via membrane filtration or other advanced treatment, to minimize pore clogging and provide higher water quality for injection into the aquifer (Page et al., 2018). As water infiltrates and travels through the subsurface, it undergoes additional treatment via filtration, adsorption, and biochemical processes depending upon the predominant redox conditions (i.e., oxic, suboxic, anoxic, or fully anaerobic) (Regnery et al., 2015b). These processes, in addition to physicochemical reactions and predation by other organisms, are thought to be primarily responsible for the inactivation of pathogenic organisms, including bacteria, protozoa, and viruses. These water quality improvements are also noted during riverbank filtration or induced bank filtration (IBF) systems, which use wells in close proximity to a river or lake to force surface water to infiltrate via an induced hydraulic gradient. However, the impact of discharged wastewater effluents in the receiving stream as well as natural fluctuations in surface water bodies can affect treatment efficacy of IBF systems (Regnery et al., 2015a). As a result, pathogenic risk management can become obscure

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and complex, resulting in approaches specifying treatment efficacy which range from generic assumptions to laborious site-specific investigations.

1.1. Pathogen removal during MAR

Due to the flexibility and nature based character of MAR systems, they have been globally applied for different purposes (UNESCO, 2021). Though the primary historic goal was groundwater augmentation, the increased usage of MAR for planned water reuse and the realization that many existing MAR applications are often part of unplanned water reuse practices have resulted in increased interest in understanding the mechanisms behind subsurface pathogen removal processes. Operators and regulators must properly manage the risk of pathogens which can potentially survive during MAR and be problematic for groundwater quality and drinking water safety. As scientific methods enabling pathogen detection and identification are continuously improving, adequately regulating and managing microbial risk in MAR systems without applying overly conservative restrictions or insufficient safety contingencies becomes a key question.

1.1.1. Assigning log reduction credits in MAR systems

Despite extensive experience in demonstrating highly efficient pathogen removal at various MAR systems, little consensus exists between scientists, MAR operators, and regulators on appropriate treatment credits for pathogen reduction assigned to a given project site (National Research Council, 2008). This uncertainty stems from the absence of commonly accepted national or international approaches for assigning pathogen log reduction credits and proper consideration of site-specific conditions. Additionally, the absence of or difficulty in obtaining full-scale data (particularly when credits must be assigned prior to commencing operation) is extrapolated by challenges inherent to the direct measurement of microbial contaminants and their unique fate and transport behavior, which is notably different than that of inorganic/organic colloids and chemical constituents (Pang, 2009). However, most existing regulations favor a rather conservative approach when assigning log reduction credits and do not address the possibility that log reduction actually achievable at a specific site could be greater.

1.2. Points of discourse in MAR characterization

1.2.1. Selection of indicators and surrogates

Over 200 waterborne pathogens affecting human health have been identified to date, whose distribution and concentrations can range seasonally and depend upon water use per capita, socio-economic factors, and occurrence of pathogens in the community (Pepper et al., 2014). Advances in molecular biology and improved detection methods have revealed greater numbers of pathogens in reclaimed water: viruses are now thought to be present in wastewater at concentrations 100 times greater than previously detected (Gerba and Betancourt, 2017). Detection methods have increased in amount and sensitivity and now include microscopic, cultural, physiological, nucleic acid, and antibody methods. However, newly detected pathogens, particularly viruses (e.g., Boca viruses, Torque teno virus, microsporidia, etc.), may exhibit unique characteristics which can prevent extrapolation of data from one viral group to another (Polo and Romalde, 2023). Recent rapid mutations of RNA viruses demonstrated the speed with which viral adaptations can occur (i.e. SARS-CoV-2) and have resulted in the first commercial test kit for detection of viral pathogens in wastewater (IDEXX, 2020). Measuring performance of MAR in terms of removal of such a wide range of pathogens is challenging in terms of the large number, different methods needed, and cost. Quantifying the removal is useful to regulatory agencies assessing MAR performance and establishing standards. The use of indicators and surrogates could fill this need and allow better assessment performance under different operational conditions including different source water qualities and other site-specific factors. Due to the broad and increasing range of detectable pathogens, selection of proper indicators and surrogates must be done carefully and include as many of the following characteristics as possible. The indicator should: 1) be present at concentrations equal to or greater than the target pathogen; 2) display equal or greater survival; 3) exhibit no seasonal variability; 4) display equal or lesser transport through media; and 5) have reasonable costs for assays or quantification. Surrogates can indicate treatment performance relative to the pathogens of interest and be used as model or index organisms to develop fate and transport models (Ashbolt et al., 2001). Despite these generally accepted requirements, comprehensive and straightforward recommendations for indicator and surrogate selection according to MAR type or source water pretreatment are currently lacking.

1.2.2. Subsurface pathogen fate and transport parameters

Selecting appropriate models for fate and transport, and ultimately risk, depends heavily on the availability of quantitative and qualitative empirical data. Viral pathogens, in particular, exhibit non-linear removal during MAR treatment (Pang, 2009) and their evolution likely affects their survival, persistence, and concentration in reclaimed water over time. Pathogen biodiversity, evolution and natural selection, and the variability of treatment process performance as well as treatment process scale additionally contribute to pathogen removal efficacy, especially in regards to MAR source water pretreatment (e.g. degree of wastewater treatment received) (Wang et al., 2022; Yuan et al., 2019). Although many fate and transport studies have been conducted at lab- or pilot-scale, transferring such results to field-scale is prone to inaccuracy, as many more variables exist at field-scale than can be reasonably tested under lab- and pilot-scale conditions. A recent study comparing removal of virus and bacterial spores (as a model for *Cryptosporidium*) found that column studies overestimated removal by two orders of magnitude compared to field removal (Oudega et al., 2021). Ultimately, internationally or nationally accepted guidelines for evaluating pathogen removal during MAR are needed.

This study reviews the guidelines and literature data on log-reduction credits applied to MAR systems to come up with a comprehensive appraisal of the pathogen removal efficacy of MAR. A new workflow comprised of key steps is proposed to determine robust removal credits for pathogens during artificial groundwater recharge based on risk management principles. This risk-based approach will enable more site-specific assessments of MAR systems considering local conditions to adequately regulate and manage microbial risk, while reducing overly conservative regulatory restrictions or insufficient safety contingencies.

2. Methods

In order to provide a comprehensive assessment of pathogen removal efficacy during MAR, peer-reviewed literature was searched for using the Web of Science, Google Search, and PubMed. Material analyzed during this comprehensive review included peer-reviewed journal articles, books, proceedings of professional meetings (e.g., International Society for Subsurface Microbiology (ISSM), International Symposium on Managed Aquifer Recharge (ISMAR)), published scientific reports, and web-based tools to estimate pathogen inactivation during MAR. When available and accessible, unpublished information from U.S. utilities and the authors was also considered. The findings were also informed by regulators, practitioners, and consultants which attended an international and a North American expert workshop in 2020 as part of the research project ‘State-of-the-Science Review: Evidence for Pathogen Removal in Managed Aquifer Recharge Systems’ funded by the Water Research Foundation (WRF).

3. Results and Discussion

This study neither examines the different requirements defined for pretreatment of water prior to injection or introduction into the subsurface, nor provides a comprehensive review on recent advances of theoretical model developments on fate and transport of pathogens, but instead focuses on current pathogen monitoring requirements in international regulations. By addressing gaps in monitoring requirements as well as in fate and transport models and approaches, an improved MAR system risk assessment strategy is proposed to assign adequate removal credits for pathogen removal.

Table 1
Pathogens and indicators used internationally for MAR and/or groundwater monitoring.

Country / End use	Virus	Coliphage	Protozoa	Coliforms	Bacteria	Microscopic particulate analysis (MPA)	Other
U.S.: Federal *(for drinking water supply)*		Coliphages (GWR[q], LT2SWTR[r])	*Cryptosporidium* (GWUDI[a], LT2SWTR)	Fecal through NPDES[b] and total through GWR	*E. coli* and enterococci[q,r]	For GWUDI	Aerobic bacterial spores (as indicators) in GWUDI
U.S.: California [c] *(at the point of exposure for indirect potable reuse project via groundwater recharge)*	≥ 12-log enteric virus		≥ 10-log each of *Cryptosporidium* and *Giardia*				Microbial, chemical or physical surrogate
U.S.: Colorado[d] *(required for determining GWUDI)*			*Cryptosporidium* and *Giardia* (case by case)	Total	*E. coli*, aerobic bacterial spores (as indicators)	Yes	
U.S.: Oregon[e]				Total (for groundwater and recycled water)	*E. coli* (for recycled water)		Allows site-specific testing to demonstrate log reduction requirements under the SWTR for IBF
U.S.: Florida[f]	Enterovirus (prior to commissioning)		*Cryptosporidium* and *Giardia* (prior to commissioning)	Fecal			Helminths (prior to commissioning)
U.S.: Virginia[g]				Fecal	*E. coli*, enterococci		
U.S.: Arizona[h], Washington[i]	Virus (only in Washington, virus unspecified)			Total			
Canada	4-log reduction (virus unspecified)[k,l]		3-log reduction of *Cryptosporidium* and *Giardia*[k]; 3-log of *Giardia*[l]				
Australia[j]	Risk assessment required to identify appropriate residence time and microbial and chemical contaminants. Guidelines and overall approach have also been adapted by India and China[m].						
The Netherlands[n,s] *(for drinking water supply)*	Enteroviruses (QMRA)	Somatic coliphages as indicator and surrogate	*Cryptosporidium* and *Giardia* (both QMRA)		*Campylobacter* (QMRA)		Fecal indicator organisms
Germany[o] *(recommended procedure)*		As indicator organisms	*Cryptosporidium* and *Giardia*		*E. coli* and enterococci		
World Health Organization (WHO)[p] *(recommended for potable reuse)*		As indicator organisms	*Clostridium* spp. as indicator organism		*E. coli* or thermotolerant coliforms, enterococci as indicators		Disinfection residuals for drinking water distribution systems

[a] = EPA Groundwater Under Direct Influence (GWUDI) definition
[b] = National Pollutant Discharge Elimination System (NPDES) | US EPA
[c] = CCR Title 22 - § 60320.108
[d] = Determination of Groundwater Under the Direct Influence (GWUDI) of Surface Water using Microscopic Particulate Analysis
[e] = OAR 340-040 (GW); 340-044 (UIC); 340-055-0012 (Recycled Water Quality Standards and Requirements)
[f] = Chapter 62.610 (IPR, GW recharge)
[g] = Chapter 740. Water Reclamation and Reuse Regulation
[h] = Aquifer Water Quality Standards
[i] = WAC 173-219-320; WAC 173-219-330
[j] = Guidelines for Water Recycling
[k] = (Alberta Environment and Parks, 2012)
[l] = (Ministère de l'Environnement et Lutte contre les changements climatiques, 2019)
[m] = (Bartak et al., 2015)
[n] = (Staatsblad van het Koninkrijk der Nederlanden, 2001)
[o] = (Umweltbundesamt, 2014)
[p] = (World Health Organization, 2017b)
[q] = (U.S. Environmental Protection Agency, 2008)
[r] = (U.S. Environmental Protection Agency, 2006)
[s] = (Smeets et al., 2009).

3.1. Regulated pathogens and indicators in MAR systems

Although many MAR operations have numerous years' worth of monitoring data (mostly focused on indicator bacteria i.e., coliforms, *Escherichia coli*), the high level of censored data (e.g. below detection limit) has perpetuated limited validation of removal and contributed to difficulties in understanding the true level of pathogen removal in these MAR systems (Donn et al., 2020). In addition, monitoring of reference pathogens has been inconsistent (i.e. different types) or has been often limited to one group or type of pathogen (e.g. enteroviruses) because of previous limitations in cost and methods. Methods for detecting pathogens in water have increased significantly in recent years, allowing detection of a broader spectrum of pathogens and lower costs (Hrdy and Vasickova, 2022). Table 1 outlines the different pathogen monitoring requirements and recommendations for MAR and/or groundwater systems in different countries.

3.1.1. The United States

In the United States, the National Pollutant Discharge Elimination System (NPDES) regulates fecal contamination via point source effluent pollution of waters, which may be source water for MAR systems only if they are discharged into surface water. The NPDES typically requires dischargers to monitor for fecal coliforms or *Escherichia coli*. However, a recent U.S. Supreme Court ruling clarified that NPDES permits are also needed for point source discharges of pollutants which reach U.S. waters after traveling through groundwater if the discharge is the functional equivalent to a discharge into surface water (U.S. Environmental Protection Agency, 2021). The Groundwater Under Direct Influence (GWUDI) concept, which applies to IBF systems where groundwater is potentially susceptible to risk from pathogens in nearby surface waters, influenced the Surface Water Treatment Rule (SWTR) (U.S. Environmental Protection Agency, 1989) via its inclusion in the Safe Drinking Water Act in 1986, which was later revised to strengthen protection against microbial contaminants (Chaudhary et al., 2009). If GWUDI is suspected, then microscopic particulate analysis (MPA) must be carried out to estimate the risk of pathogen intrusion, although MPA was not intended to assess the fate and transport of human health relevant waterborne pathogens. IBF wells suspected to be GWUDI can demonstrate removal of *Cryptosporidium* to receive credits greater than what is allocated by the Long-term 2 Surface Water Treatment Rule (LT2SWTR) (U.S. Environmental Protection Agency, 2006). Recent efforts have focused on identifying indicators of pathogen occurrence, as well as pathogen surrogates which are diverse in structure, morphology, and culture, and on developing sampling concentration and detection protocols for adequate yet conservative application for GWUDI settings. However, pathogens analyzed under GWUDI vary by state. Additionally, aquifer recharge operations may also require additional aquifer protection permits to be obtained (e.g. Arizona).

In the state of California, only recycled water having undergone tertiary treatment and disinfection or advanced water treatment can be used as recharge water in indirect potable reuse projects (California Department of Public Health, 2014). Obtaining log reduction credits for pathogens can be done via a challenge test and providing evidence of reliable and consistent treatment. Monitoring of the reference pathogen, as well as a microbial, chemical or physical surrogate, is required to ensure each unit treatment achieves its allocated log reduction. In subsurface applications, 10-log protozoa reductions can be achieved when disinfected tertiary effluent or advanced treated effluent undergoes at least 6 months of subsurface retention time. Each month of subsurface retention time is only credited with 1-log of virus reduction, as decay rate under constant environmental conditions is irregular.

In the state of Colorado, in addition to the typical indicators included in the MPA method, the Colorado Department of Public Health & Environment requires analysis of aerobic bacterial spores, which were suggested as a valuable addition to GWUDI assessment indicators (Abbaszadegan et al., 2011). Aerobic spores could be a useful surrogate in GWUDI assessments and for public health protection in addition to total coliforms, *Escherichia coli*, enterococci, and coliphages currently used in the Ground Water Rule (GWR) (U.S. Environmental Protection Agency, 2008) and the LT2SWTR (U.S. Environmental Protection Agency, 2006). While the state of Texas also has active MAR facilities, it does not specify log reduction allocations or pathogen monitoring.

3.1.2. Canada

The absence of federal regulations for MAR systems in Canada has prompted individual provinces to develop their own regulations. MAR systems in Canada are primarily GWUDI IBF systems. Some provinces have no official rules for GWUDI assessments, while others (e.g., Ontario (Ontario Ministry of the Environment, 2001), Quebec (Ministère de l'Environnement et Lutte contre les changements climatiques, 2019; Patenaude et al., 2020), Alberta (Alberta Environment and Parks, 2012)) have developed very detailed regulations and guidelines.

Ontario's GWUDI system paradigm uses a different approach than that of the U.S. Specifically, Ontario does not prescribe requirements for treatment credits for pathogen removal and log removal targets based on subsurface travel time, as this did not seem defensible given that the distribution of various subsurface travel times may apply to chemical tracers but not to particle transport. The actual maximum subsurface travel time for pathogens was considered to be uncertain and variable, given the dynamic hydrological conditions in GWUDI systems and heterogeneous aquifer conditions, and therefore travel time was not considered to be an adequate surrogate for public health protection. Instead, regulations require direct monitoring of well water for key water quality parameters for public health protection. Monitoring programs focus on possible baseline water quality changes in wells and set stringent alert levels, enabling detection of such changes. In this way, Ontario focuses more on direct exposure measurements by well water quality monitoring and by defining post-treatment requirements of the recovered water and less on what may occur in the subsurface.

3.2. Australia

Regulations in Australia targeting the augmentation of drinking water supplies with recycled water and the practice of MAR have been in place since 2008 (NRMMC–EPHC–AHMC, 2008; 2009). The guidelines require a risk assessment (both maximal and residual risk) of each project to account for different end uses and site-specific conditions, including appropriate retention time, and as a consequence, identification of site-specific microbial and chemical contaminants (Dillon et al., 2022). The minimal subsurface retention time for drinking water production is required to meet $2{\times}10^{-6}$ disability adjusted life years (DALYs) per person per year (NRMMC–EPHC–AHMC, 2008; 2009), but retention time can vary for reuse schemes with less stringent requirements (e.g., urban reuse vs potable reuse). Compared to the approach of certain U.S. states, the Australian guidelines are less prescriptive, and rather emphasize the site-specific risk assessment process for developing performance-based outcomes for public health protection. The Australian guidelines have also been adapted by India and China (Bartak et al., 2015).

3.2.1. Europe

Although there is no regulation at the level of the European Union (EU) on indirect potable reuse schemes beside the EU Groundwater Directive (European Commission, 2006), several member states (e.g., Spain, France, Italy, Cyprus, Greece, Portugal, Malta) have developed their own reuse standards or groundwater recharge operation regulations (Fawell et al., 2016; Rebelo et al., 2020; Yuan et al., 2016). All EU member states are additionally required to comply with the European Drinking Water Directive in their national drinking water legislation (European Commission., 2020a). The recently passed Regulation (EU) 2020/741 on minimum requirements for agricultural water reuse, which will go into effect in June 2023, requires monitoring of *Legionella* *spp.* and intestinal nematodes, as well as demonstrated log reduction of coliphages, *Clostridium perfringens* spores, spore-forming sulfate reducing bacteria, and *Escherichia coli* (European Commission., 2020b).

In the Netherlands, utilities must demonstrate that enteric viruses, *Campylobacter, Cryptosporidium* and *Giardia* and any other pathogen in the treatment systems are removed prior to groundwater recharge to fulfill the maximum acceptable annual infection risk from pathogens of 1 per 10,000 inhabitants in drinking water (Staatsblad van het Koninkrijk der Nederlanden, 2001). At least 60 days of retention time of groundwater is required for preventing contamination near drinking water wells, and monitoring requirements of fecal indicator organisms must also be met (Smeets et al., 2009). Somatic coliphages are the recommended indicators for virus transport due to appearing at 1,000-10,000x greater concentrations than enteroviruses. Enteroviruses are considered the most relevant MAR index pathogens due to their high infectivity, small size, rather poor attachment to sand (the prevalent geological material in the Netherlands) due to low organic matter content and incompatible charge, and slow inactivation. Well monitoring requirements focus on fecal indicator organisms (Smeets et al., 2009).

Germany requires a minimum of 50 days of subsurface retention time as well as monitoring of indicator organisms to protect drinking water from bacterial contamination. A recommended quantitative microbial risk assessment (QMRA) procedure published in 2014 by the German Federal Environment Agency for MAR and other groundwater systems suggests regular sampling (of at least 12x a year) for *Escherichia coli* and enterococci, *Cryptosporidium* and *Giardia*, and indicator coliphages (Umweltbundesamt, 2014). This has begun to be implemented by utilities and will be monitored to determine how successfully pathogenic risk can be mitigated. Switzerland requires a minimum retention time of 10 days and a minimum distance of 100 meters in the subsurface to achieve proper removal of bacteria and viruses (Der Schweizerische Bundesrat, 1998). Austrian regulations require a subsurface travel time of 60 days for groundwater abstraction (Zetinigg, 1995). Hungarian law requires a minimum of 20 days travel time or a minimum of 10 m radius for abstraction wells (Ministry for Environment and Water, 2006).

3.3. Up-and-coming indicators and surrogates for pathogen assessment

The number of waterborne pathogens of human health concern detected in domestic wastewater continues to grow as detection methods improve. Table 2 presents a non-comprehensive overview of those detected to date.

Indicators and surrogates should be selected based on the target pathogens of interest at a particular MAR site. Although *Escherichia coli* is often monitored, there is rarely a direct correlation with other human pathogens (Pepper et al., 2014; World Health Organization, 2017a). As viruses are more difficult to remove in MAR systems than bacteria or protozoa because they are significantly smaller in size and survive longer, their absence or significant reduction ensures that bacteria and protozoa have also been significantly reduced or removed to below detection limit. Therefore, selecting viruses as a measure of MAR pathogen removal performance in terms of pathogen removal credits or treated water quality may be acceptable.

Adenovirus has been suggested as a potential indicator because it occurs in greater concentrations than other human enteric pathogens and shows little seasonal variation (Yuan et al., 2016). Enteroviruses, which have often been monitored at MAR systems due to the existence of cell culture and infectivity assays, still require special laboratories as well as long processing times for detection (Pepper et al., 2014). However, newer indicators for viruses such as pepper mild mottle virus (PMMoV) and CrAssphage, which, in addition to being used for quantifying virus removal in WWTPs due to year-round presence (Farkas et al., 2020; Kitajima et al., 2014; Shirasaki et al., 2018; Symonds et al., 2018), are more resistant to removal during MAR than the human pathogenic viruses (Betancourt et al., 2014; Betancourt et al., 2019; Morrison et al., 2020;). Therefore, selecting groups of surrogates or indicators, such as coliphages (e.g. MS-2), plant viruses (e.g. PMMoV) and/or groups of viruses (e.g. CrAssphage) can be used to assess the overall performance of MAR. Other non-pathogenic organisms such as dyed bacteria or even particles (e.g., latex microspheres, coated silica nanoparticles) can also be used as surrogates for pathogen removal (Clemens et al., 2020; Harvey et al., 1989). However, they may not accurately represent the behavior of biological entities like bacteria and viruses, which are neither uniform in size nor in chemical surface composition.

An analysis of the most commonly used coliphages in column and field studies to model virus survival and transport, which included MS-2, PRD-1, ΦX174 and the F-specific RNA bacteriophages (FRNAPH coliphage), concluded that FRNAPH, as a group of naturally occurring viruses in wastewater, are very useful model viruses for viral subsurface transport behavior (Schijven and Hassanizadeh, 2000). FRNAPH not only behave relatively conservatively (e.g. similarly to MS-2) and are very persistent in the environment, but naturally present FRNAPH are also poorly adsorbed during soil passage treatment (Schijven and Hassanizadeh, 2000). Although somatic coliphages are present in larger numbers than FRNAPH, FRNAPH are more homogeneous in size and shape.

When subsurface residence time is shorter (e.g. IBF), aerobic spores, together with total coliforms, can be used as surrogates for *Cryptosporidium* (Berger et al., 2018). In groundwater systems, flow cytometry has recently received attention as an alternative surrogate measurement for pathogen transport (Safford and Bischel, 2019). However, qPCR is more sensitive for virus detection than flow cytometry, providing further support for more widespread acceptance of qPCR. The use of chemicals as surrogates (e.g., nitrates, sucralose, primidone, etc.) must be carefully considered, as the different mechanisms governing colloidal versus chemical transport in aquifers can under certain conditions lead to overestimation of viral residence time when relying solely on chemical tracers (McKay et al., 1993). Table 3 provides a non-exhaustive list of microorganisms and compounds which can be used as indicators and/or surrogates to assess pathogen inactivation in MAR systems.

By applying both conservative virus surrogates (e.g. coliphages) and non-conservative surrogates (e.g. coated silica nanoparticles), safe and realistic separation distances for wells in saturated aquifer systems may be estimated (Clemens et al., 2020; Wang et al., 2022).

Table 2

Size and examples of pathogens of human health concern detected in municipal wastewater (adapted from (Pepper et al., 2014)).

Protozoa (several μm)	Bacteria (≥ 1 μm)	Viruses nm		Indicator / Surrogate viruses
Cryptosporidium spp.	*Salmonella spp.*	Rotavirus (60-80)	Achi virus (23)	MS2
Giardia lamblia	*Campylobacter spp.*	Adenovirus (70)	Parvovirus (18-23)	Qβ
Entamoeba histolytica	*Shigella spp.*	Norovirus (23-40)	Circovirus (15-22)	ΦX-174
Cyclospora cayetanensis	*Yersinia*	Astrovirus (28-35)	Bocavirus (18-26)	FRNAPH
Toxoplasma gondii	*Vibrio spp.*	Hepatitis A and E (27-34)	Saprovirus (27-49)	PMMoV
Microsporidia	*Pathogenic E. coli*	Enteroviruses (Coxsackie, Echo) (23-30)		CrAssphage
Toxoplasma gondii	*Listeria spp.*			Bacteroides phage
				Somatic coliphages

Table 3
Considerations for deciding on appropriate surrogates and indicators for MAR systems (Betancourt et al., 2014; Kitajima et al., 2014; Morrison et al., 2020; Schijven et al., 2016; Schijven and Hassanizadeh, 2000; Symonds et al., 2018; Tandukar et al., 2020).

Microorganisms / Compounds	Advantages	Limitations
Somatic coliphages and male specific RNA coliphages (FRNAPH) (indicator, surrogate) *Variable size*	Surrogate for viruses in above ground treatment Surrogate for viruses in subsurface Infectivity can be measured via culturable assays Already in the California water recycling and Dutch challenge spiking test requirements Direct relevance to human health (gastrointestinal illness)	Lower concentrations than plant or certain bacterial viruses If used as surrogate: need to consider shape, size, presence of envelope 10-12 taxonomic groups of coliphages with varying size and stickiness require careful selection
Bacteriophage MS2 (indicator, surrogate) *Variable size*	Suitable surrogate for field spiking tests Effective removal can be demonstrated even at short distances (~10 feet)	
CrAssphage (indicator) *Variable size*	Smallest known size of all viruses Low seasonal variability Indicator for viruses in above ground treatment Present in high concentrations	Can only be easily detected by qPCR
Adenoviruses (indicator, surrogate) *(70 nm)*	Resistant to above ground treatment, disinfection Conservative indicator for viruses in above ground treatment Conservative indicator for viruses in subsurface Prevalent in source water Good indicator for systems with short HRT (e.g. IBF) Low seasonal variability	Free DNA may persist for long in the environment Largest enteric virus
Enteroviruses (indicator, surrogate) *(23-30 nm)*	Well-developed infectivity assays and detection methods Many studies on transport through soil conducted	Concentrations in wastewater vary seasonally Represent only a small fraction of all the human enteric viruses detectable in wastewater
Rotavirus *(60-80 nm)*	Suggested by the WHO Good indicator for systems with short HRT (e.g. IBF)	Not present as often as other viruses
Aichi viruses (indicator) *(23 nm)*	Infectivity assay available Limited seasonal variation	Little is known about transport through soil
Pepper mild mottle virus (PMMoV) (indicator, surrogate) *(17×312 nm)*	Highest concentrations in wastewater year-round, low seasonal variability Most abundant RNA virus in human feces Conservative indicator for viruses in subsurface and surrogate for virus in above ground treatment Indicator for preferential flow paths High concentrations detectable in MAR sites	Can only be easily detected by qPCR Use as indicator/surrogate overly conservative due to persistence Too conservative for QMRAs
Silica beads covered with virus specific proteins	Designed to closely simulate fate of viruses Utilized in New Zealand	
Cryptosporidium and *Giardia*	High infectivity	Infrequently present in source water Not detectable in MAR with longer residence time Too large for use as surrogates
Algae	Successfully used as spiking surrogate in O3/BAF systems Small size allows usage as virus proxy	Ubiquitous occurrence, not necessarily causing contamination
Aerobic spores or anaerobic spores (Clostridium)	Aerobic spores are reliable surrogates for IBF Persistent Anaerobic spores index organisms for Australian MAR systems	Possibly overly conservative for *Cryptosporidium* due to ubiquitous occurrence and smaller size Vary in size Selection tricky due to wide range of spores present in source water and detection limit of source water type
Fecal DNA markers (HF 183, HF 182)	Sensitive detection method	Poor surrogate for *Cryptosporidium* survival Nucleic methods are generally overly conservative for use as indicators, detection may not pose public health threat
Microsporidium	Now considered related to fungi	
Plastic microspheres, Free DNA, DNA encapsulated in polymers	Possess attractive surrogate properties	Application is rather size dependent (site-specific, column vs field scale)
Chemicals (e.g., persistent chemicals of concern, PFAS, isotopes, etc.) (surrogates)	Low cost detection methods available Well known, often utilized Can be detected in groundwater Useful for determining subsurface travel time in the field Useful as tracer tests for determining placement of monitoring wells	Limit of detection higher than for organisms Transport likely slower than pathogens due to pore size exclusion of viruses Solute transport is different than colloid transport

3.4. *Pathogen toolbox approach for selection indicators and surrogates*

MAR operators are recommended to diversify the organisms they monitor depending on the pathogens of concern and the site conditions. Adapting a toolbox approach, which encourages MAR systems to select the most appropriate pathogens and consider all classes (viruses, protozoa, bacteria), depending on multiple factors (above ground vs subsurface treatment, type of MAR system and aquifer, scale, selection of surrogates for removal versus inactivation), would help to characterize fate and transport at all scales (Wang et al., 2022). Furthermore, sorting viruses into different bins based on relevant properties and fate parameters would also inform selection of appropriate indicators and surrogates.

The increasing number of viruses and bacteria exhibiting resistance to disinfection makes monitoring their transport a higher priority, and could be covered by including male-specific and somatic bacteriophages as monitoring surrogates in IBF and routine groundwater monitoring. The appropriate level of conservatism should be selected by choosing the surrogates and indicators which will neither eliminate MAR from consideration nor make MAR implementation too costly. Ensuring that the range of the occurrence concentration and analytical method sensitivity enables validation of the highest required removal rates is critical. Site-specific approaches should, however, be weighed against the need for regulatory consistency within a certain radius (e.g., state, region) to avoid confusion and misinterpretation.

3.5. *Current understanding of pathogen fate and transport*

The following is a non-exhaustive list of parameters affecting pathogen removal in the subsurface, which can be classified into three main groups: operational (infiltration rate, vadose zone depth, subsurface retention time, velocity, dynamic water flow regimes and water quality changes, temperature and climate); physicochemical and water quality (organic matter in MAR source water and soil, physicochemical soil characteristics, hydrophobicity, hydraulic conductivity, redox conditions, adsorption, filtration, level of wastewater pretreatment, isoelectric points of viral pathogens, ionic strength of source water, and nitrogen, along with other water quality parameters); and biological (die-off or decay rate, predation, microorganisms present in the water and subsurface).

Typically, microbial removal follows a biphasic pattern, with initial rapid removal or decay followed by a less steep removal or decay (Pang, 2009). The infectivity is thought to decrease with increasing retention time, and protozoan infectivity is reduced faster than viral infectivity (Sidhu et al., 2015). Inactivation also increases near the soil surface and vadose zone, which are characterized by changing temperature and moisture content affecting predation and biological activity. Pathogens which are immobilized may remobilize after changes in water quality or flow occur (Masciopinto et al., 2008; Quanrud et al., 2003).

However, few studies have successfully determined fate and transport parameters at field-scale, and studies covering the wide spectrum of redox conditions (among other parameters) are also lacking. Research has either focused on pathogens/indicators which can be easily detected and analyzed (e.g., *Escherichia coli*, coliforms), regulated pathogens (e.g. *Cryptosporidium* for surface water in the U.S.), or on pathogens recommended by the WHO for monitoring in drinking water quality (e.g., rotavirus, *Cryptosporidium, Campylobacter)* (World Health Organization, 2017a). However, as viruses are of the greatest concern in MAR systems due to their small size and long-term persistence (Betancourt et al., 2014), perhaps more attention should be paid to selecting appropriate indicators and surrogates for them. In addition, viral analytical methods are the most sensitive available (one virus can be detected in 100-1,000 liters of water) (Hunt et al., 2010). The interplay between the aforementioned parameters responsible for pathogen removal at each MAR location, along with changes in the balance of these parameters, requires high resolution knowledge of the location to determine which microorganisms or contaminants listed in Table 2 and Table 3 are relevant for monitoring and risk management.

3.5.1. *Obtaining fate and transport values*

Removal rates ($\log_{10}$ removal of pathogen per day) and inactivation rates ($\log_{10}$ decline of pathogen per day), represent removal (adsorption, etc) and inactivation (die-off, decay) observed at lab-, pilot-, and field-scale MAR studies, are notoriously sparse in literature, and vary from site to site. Most studies reporting removal or inactivation rates of pathogens are over 10 years old (Gordon and Toze, 2003; John and Rose, 2005; Sidhu and Toze, 2012), with some rates obtained in the late 1980s still used for modeling predictions today (e.g., polio virus (Jansons et al., 1989; Yates et al., 1985), MS-2 (Yates et al., 1985)). Understandably, as results of inactivation and removal rates for newer pathogens are sparse, modelers are left with little choice but to work with literature values often obtained under different redox, temperature and operational conditions than what is relevant for their systems. Most inactivation or removal rates were acquired under controlled one-dimensional (1D) lab-scale experiments (e.g. column studies) or 3D tank studies, due to their low cost and regulatory acceptance (1D studies) and simulation of flow pathways and heterogeneities (3D studies). However, lab-scale studies have been shown to overestimate field-scale removal by as much as 2-3 orders of magnitude (Pang, 2009; Regnery et al., 2017), attributed to the fairly homogeneous nature of column studies, even when using material sampled from the field, as well as the simplified experimental setup of columns compared to the numerous dimensions, parameters, and flow regimes present at field-scale (Hornstra et al., 2018; Liu et al., 2016; Sidhu et al., 2015; Torkzaban et al., 2019). 3D studies are plagued by many of the same problems as 1D studies, primarily use a few homogenous strains of organisms which are easily grown in the laboratory, and can still underestimate microbial survival compared to field studies. Changing operational conditions such as pumping rates from recovery wells can influence removal, with lower removal exhibited at greater pumping rates (Oudega et al., 2022).

In situ determination of pathogen survival at field-scale is useful for determining long-term removal. Such methods, however, result in a conservative assessment, particularly when using dialysis bags/chambers with membranes, which prevent passage of antimicrobial microorganisms and exclude other organic solutes and bacteria based on pore size (Regnery et al., 2017). The lack of predation, adsorption, and filtration processes affecting pathogen removal when using *in situ* chambers contributes to a conservative estimation (Sidhu et al., 2015). However, conducting controlled tests using actual recharged water has confirmed benefits: *Escherichia coli* cells were recently shown to adapt to the water matrix they are in, which can alter the physicochemical properties of bacterial cells (e.g., zeta potential, hydrophobicity) and their transport behavior and consequently inactivation and overall removal in media (Fan et al., 2020).

At field-scale, soil structure (e.g., macropores, heterogeneity) impacts microbial removal more than soil texture (e.g. how fine/coarse a soil is) (Schijven et al., 2016). Identifying the dominant soil type or the soil mixture will facilitate better prediction of which pathogens can be best removed in the soil. If little is known about the soil properties, taking a removal rate determined from diffuse pollution over long-term effluent loading would constitute a conservative assessment (Schijven et al., 2016). If soil characteristics are known, options to increase removal and predict removal rates could be identified using simple 2D models based on the schematic of the MAR facility coupled with reactive transport equations and empirical knowledge. This was done in recent work, where transport was modelled using colloid filtration theory and HYDRUS model (Pang et al., 2021). An overview of colloid facilitated contaminant transport can be found elsewhere (Deb and Chakma, 2022).

Observation of monitoring and pumping wells is important for providing a more accurate assessment of pathogen removal (Morrison et al., 2020). Monitoring wells may represent a much smaller area from which the water is withdrawn, since they are typically located in close proximity to the infiltration location of either the vadose zone in the case of dry wells or the saturated zone of an aquifer. Pumping or production wells can draw water from a larger area with a higher rate. Therefore, combining assessment of both types of wells will more comprehensively reflect the occurrence of indicators and surrogates. Such dual monitoring would also help account for lenses of different porosity, which may result in the development of saturated zones and influence greater subsurface transport of microbes (Powelson et al., 1993).

To date, many field-scale studies have focused on human enteroviruses and coliphages (Regnery et al., 2017), which may not be the most appropriate surrogates, as enteroviruses occur in lower concentration than other enteric viruses (e.g. adenovirus) (Gerba et al., 2017). Additionally, viral removal under saturated and unsaturated conditions has been shown to differ (Zhang et al., 2021; Zhunag and Y., 2003), and numerous research gaps in determining pathogen removal under unsaturated conditions remain. Testing for other groups of viruses in greater abundance and which survive longer in the environment than enteroviruses should be conducted.

3.5.2. *Appropriate modeling strategies for predicting fate and transport depending on setting*

Initial fate and transport models incorporated first-order inactivation rates of free and attached viral particles in the subsurface, as well as reversible adsorption and different time-dependent inactivation rate coefficients (Chrysikopoulos and Sim, 1996; Sim and Chrysikopoulos, 1996). More recent modeling work has improved predictive abilities by modeling virus transport in multiple aquifer layers of different hydraulic conductivities in aquifer storage and recovery using the infinite element domain feature of the COMSOL Multiphysics software (Torkzaban et al., 2019). Such models could be further improved by incorporating additional removal mechanisms for viruses, such as attachment, detachment and solid phase inactivation (Torkzaban et al., 2019). If incorporated, more accurate predictions of necessary residence times for solid phase inactivation can be estimated to reduce post-treatment costs for MAR recovered water. Other approaches have begun to include the use of machine learning to describe microbial transport in porous media (Ke et al., 2022). Additional modeling approaches can be found in (Zhang et al., 2022). Recent work also studied and modeled the effect of seasonal changes on transport of viruses and indicators in induced bank filtration using PFLOTRAN to create a 2D groundwater model (Knabe et al., 2023).

However, uncertainty in modeling and scale-up of results to pilot- or field-scale applications remains, in part attributed to heterogeneities in the subsurface (Masciopinto et al., 2008; Toze et al., 2010). Heterogeneities and preferential flow paths could be better characterized by conducting full-scale tracer tests with higher resolution sampling along the entire pathway of the MAR scheme instead of pump tests which are typically used to define hydraulic characteristics (Toze et al., 2010). Such increased resolution accuracy would positively impact the modeling of pathogen transport to drinking water wells and illustrate the influence of preferential flow paths in field-based research, in turn facilitating more accurate risk assessments (Bradford and Harvey, 2016). This would, however, require discussion prior to construction, and could be more difficult to implement at certain (smaller) locations if a MAR facility is already operational. Additional benefits arise when using spatial imaging to determine where to locate recharge facilities (Alam et al., 2022), using remote sensing (satellite radar altimetry) to correctly place or detect wells (Houben et al., 2019), or gathering data from online sensors to assess the state of the system at higher resolution (Sánchez-García et al., 2019). If short residence times characterize the MAR system (e.g. IBF), an online decision support tool developed by the German Federal Environment Agency can be used to determine occurrence of viruses in bank filtrate and select appropriate pathogens for further monitoring (Link). This approach can be used when extensive data for modeling do not exist. However, testing for pathogens still provides the most site-specific and informative results and is likely the less costly approach in comparison to modeling. With lab costs down to about $350-$500 USD per sample using qPCR, much can be learned from sampling even ~10 times per year. Methods are continuously being improved, for example through the incorporation of smartphone-based fluorescence microscopes, which have been proposed for detecting single virus copies (Chung et al., 2021).

Approaches using probabilistic models for targeted health-based risk modeling can be utilized for initially estimating removal ranges, which can identify pathogens for which removal during MAR should be optimized and determine setback distances for extraction wells (Blaschke et al., 2016). Such approaches could include quantitative microbial risk assessment (QMRA). This can circumvent certain challenges when accounting for spatial changes in groundwater flow and parameters controlling pathogen retention and field-scale heterogeneity, although knowledge on parameters such as size exclusion, reversibility of retention, release of pathogens, and field-scale flow, transport, and fate processes remains low (Bradford and Harvey, 2016). Panagiotou et al., 2022 used a QMRA approach for setting health-based performance targets for soil-aquifer treatment of wastewater. They concluded that greater removal of rotavirus and *Cryptosporidium* was required than *Escherichia coli* to meet their desired target.

3.6. *Proposed workflow to predict log reductions for full-scale systems*

Current regulations and policies favor conservative assumptions for removal of viral, but also protozoan and bacterial, pathogens in the subsurface. This has stemmed in large part from the difficulty of obtaining full-scale data and direct log reduction measurements, as detection of pathogens in low concentrations using cost-effective sampling and detection methods has only recently become more widely accessible. However, the advent of higher sensitivity detection methods (Jahne et al., 2020; Kojabad et al., 2021), in addition to higher resolution fate and transport models and protocols for conducting tracer tests, should prompt the reassessment of assumptions of guidelines.

Therefore, expanding beyond the most commonly required total or fecal coliforms or *Escherichia coli* monitoring to increased monitoring of coliphages or other indicators and surrogates could be beneficial for many MAR systems and also improve public health protection. The appropriate selection of surrogates and models for assigning log reduction credits must be determined on a case-by-case basis, considering the operational, physicochemical, water quality and biological parameters affecting pathogen removal. Assigning log reduction credit and performance removal using surrogates and empirical data can be recommended for MAR systems showing high degrees of heterogeneity.

This paper recommends a 10 step strategy for demonstration sites (Fig. 1). In step #1, MAR operators would first be obliged to fulfill the basic regulatory and monitoring requirements of their responsible regulatory body, as different countries have different requirements. All subsequent steps (#2-#10) are optional methods which operators could use to prove greater log reduction of pathogens than what is granted by default. By characterizing source water quality (using more sensitive detection methods) as well as the site (via high resolution data acquisition), operators could determine which pathogens, and in turn which surrogates and indicators, are most suitable for monitoring. Conducting a critical control point analysis can identify where potential pitfalls are, as well as the technically assessible unit treatments. Afterwards the operators can determine whether lab-scale or *in situ* field testing is feasible, according to the benefits and shortcoming of both approaches (i.e., cost, assumptions taken, etc.). If *in situ* testing is feasible, fate and transport models can be set up, calibrated, and ultimately validated by obtained data. Finally, risk assessment models can be used to adequately characterize, manage and mitigate risk. Following the Dutch or Australian risk-based approaches, which already include some of these suggestions, could also reveal a more tailored and perhaps more cost-effective monitoring plan for each MAR system interested in obtaining more removal credits. Such an approach would ensure regulatory compliance of all MAR systems, but would enable individual systems to provide evidence for permitting decisions, utilize the subsurface removal processes to the greatest capacity possible, and ultimately reduce post-treatment costs.

4. Conclusions

Findings of this study stress that it is time to reassess the science behind current regulations for affirming adequate pathogen removal in MAR systems. Improved molecular detection and microbial analysis techniques have identified increased quantities and types of waterborne pathogens. As more planned water reuse systems practicing groundwater recharge come online in the next years due to increasing water shortages, improving the assessment of pathogen removal in MAR systems could be beneficial in terms of maximizing removal potential and cost savings.

By using the 10 step approach discussed in this paper, MAR operators can meet local and regional water quality and public health goals while also following a systemic assessment approach to verify the pathogen removal actually attainable at their site. Testing source water for a portfolio of pathogens using the toolbox approach to determine which are most relevant for each individual MAR system can narrow down the

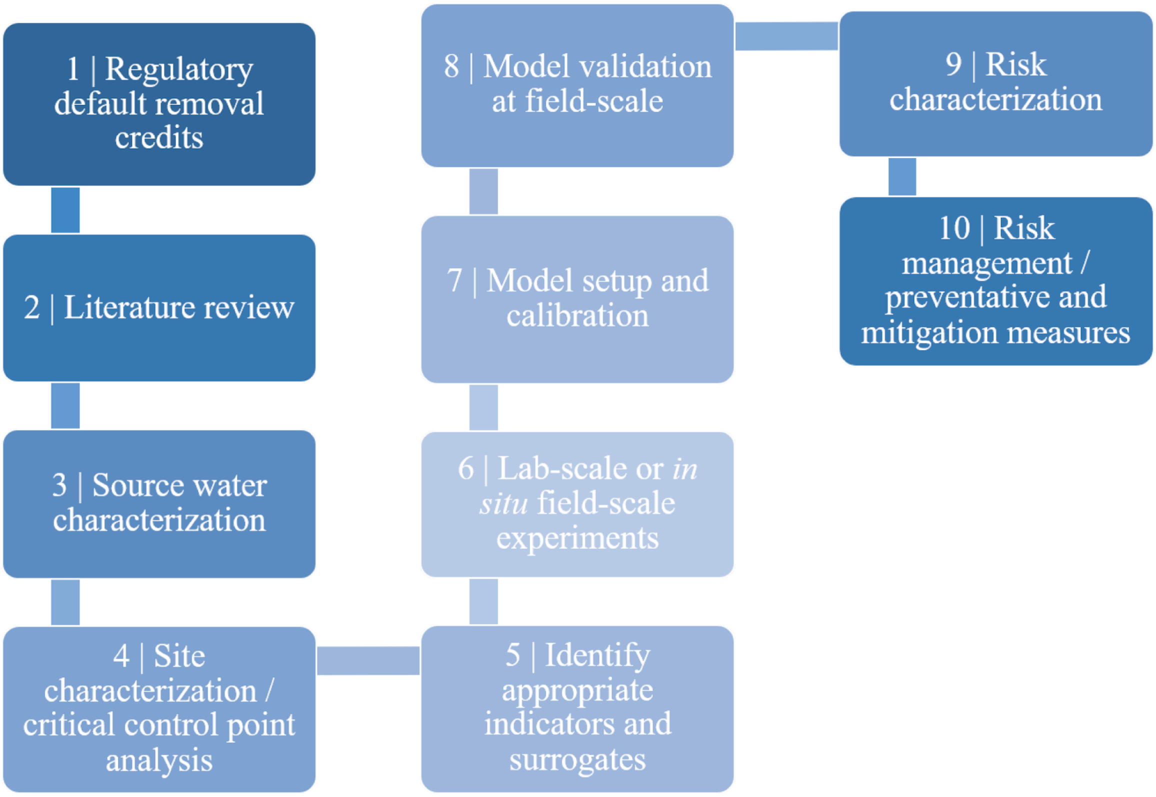

Fig. 1. Proposed workflow for demonstrating site-specific pathogen removal credit at groundwater recharge locations. Variances can be requested after steps 2, 5, 8, and 9.

costs of monitoring and identify the most appropriate indicators and surrogates for local pathogens of concern. Consistent removal of these pathogens would assure regulators that MAR systems can safely and reliably provide desired final water quality. Then, if the individual site can and is interested in demonstrating and being accredited more log reduction credits, how many and which steps to take to improve their removal can be decided upon. Using such a risk-based and tailored approach would enable an individual assessment of each MAR system, allowing adequate management of risks in indirect potable reuse MAR systems without overly conservative restrictions or insufficient safety contingencies.

Numerous topics should be explored in future research. The suitability of surrogates and indicators could be investigated via a meta-analysis of decay or die-off rates to determine whether correlations with certain redox conditions exist, and to refine rates which were obtained many years ago but are still in use. Metagenomics can be used for characterizing and comparing microbial populations involved in pathogen removal at both column- and field-scale, and whether spores are suitable surrogates for protozoa removal at SAT or ASR sites should be assessed. Opportunities for new surrogates and indicators should likewise be investigated: silica beads with virus-specific proteins; free DNA or RNA encapsulated in polymers; online flow cytometry, or DNA binding dyes to detect viability in PCR; metagenomics and/or adenosine triphosphate (ATP) can be used to define optimal MAR operation for pathogen removal by monitoring organisms relevant to the removal of indigenous organisms. New surrogates can be used as conservative indicators of human pathogenic viruses (e.g., PMMoV, CrAssphage). Monitoring pathogen and indicator removal in real time can now be accomplished through the use of digital droplet PCR (ddPCR) even prior to the SARS-CoV-2 pandemic (Jahne et al., 2020), which could be applied at other types of MAR systems (e.g., dry wells, stormwater infiltration). Additional attention should be paid to developing and publishing fate and transport models attuned to the specifics of IBF systems, addressing release pulses as well as attachment and detachment processes. Finally, the recommendations developed in this paper should be tested at full-scale sites to determine guidelines and best management practices for regulatory consideration.

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.

Data availability

No data was used for the research described in the article.

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