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Review

A biotechnological perspective on sand filtration for drinking water production

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 Mark CM van Loosdrecht and Doris van Halem



Gravity-driven sand filters are the dominant groundwater treatment technology for drinking water production. In the past, physicochemical reactions were often assumed to play the main role in the removal of contaminants, but recent breakthroughs showcase the vital role of microorganisms. In this *Current Opinion*, we thoroughly assess the current understanding of biology in sand filters and explore the potential benefits of shifting toward designs aimed at promoting biological reactions. We highlight the main bottlenecks and propose key areas to be explored toward the next generation of sustainable, resource-efficient groundwater biofilters.

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The complexity behind seemingly simple groundwater biofilters

The sequence of aeration followed by gravity-driven filtration¹ has been the dominant groundwater treatment

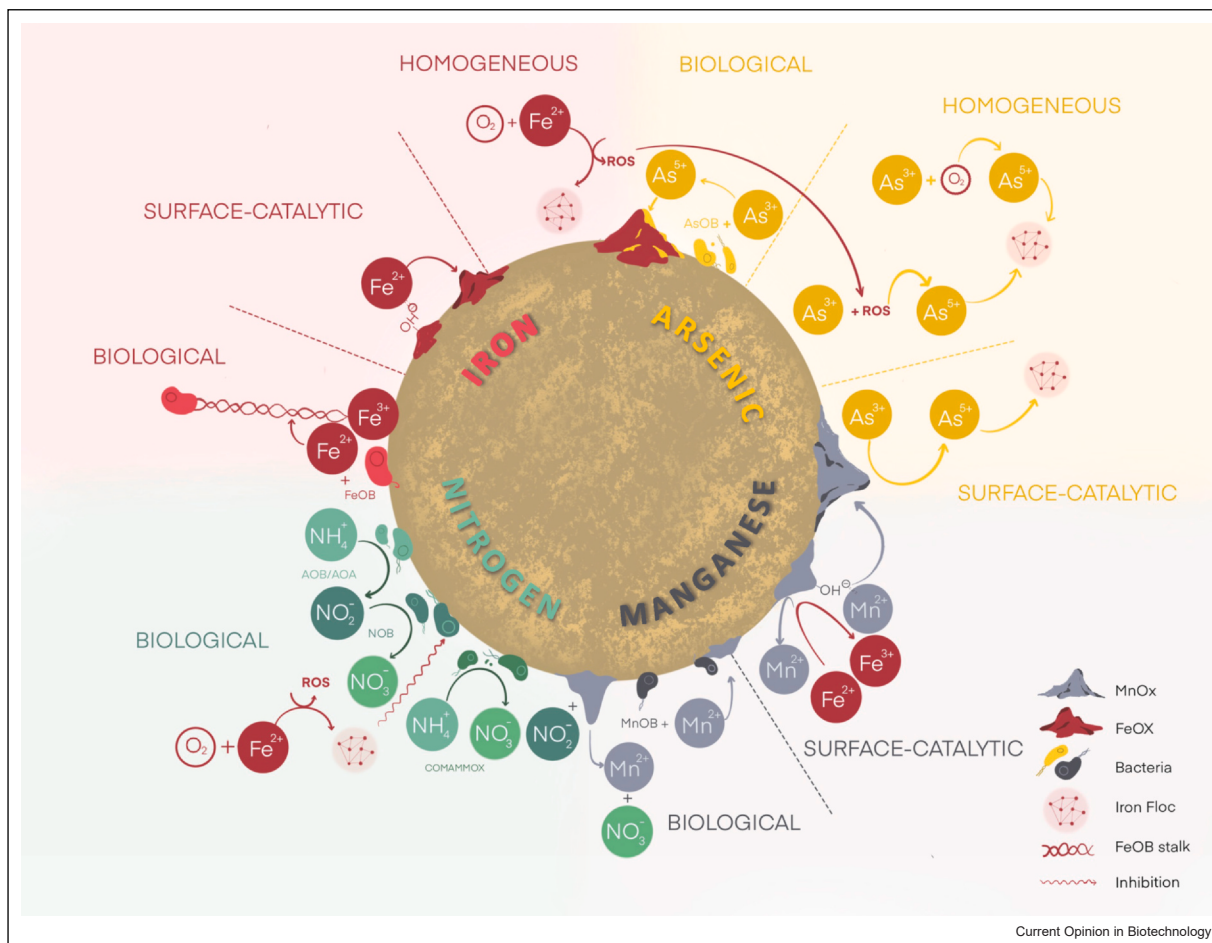
¹ Groundwater filtration is most often performed with granular media, known as granular filtration. Several types of matrices, such as quartz sand, anthracite, or pumice, are used in granular filtration. Among them, sand quartz is the one used in the vast majority of cases in full-scale filters, thus the most commonly used in research. As a result, *sand filtration* is the term commonly used to refer to all types of granular filtration. For consistency, we use the term (*sand*) *filtration* throughout this opinion article but refer to all types of granular filtration.

technology for centuries. Relatively simple designs and operational conditions yield reliable and robust systems for the removal of the main anaerobic groundwater contaminants, namely, iron, ammonia, manganese, and arsenic. However, behind its seemingly trivial working principles, sand filters harbor a high degree of complexity. The introduction of oxygen to saturation levels into anaerobic groundwater onsets a sophisticated network of simultaneous, interconnected, and uncontrolled physicochemical and biological reactions (Figure 1). Additionally, the continuous accumulation of iron and manganese deposits within the sand filter forces practitioners to backwash the sand filter, perturbing its steady state and creating a dynamic system. This inherent complexity makes both research and process optimization challenging. As a result, sand filters have traditionally been considered as a *black box*.

During the last decade, the implementation of new molecular tools, in particular metagenomics [1] and metaproteomics [2], substantially increased our understanding of sand filters. The central role of microorganisms has been recognized, and sand filters are now commonly referred to as *biofilters*. However, research is often limited by the difficulty to access the biofilm, intricately entangled with metal oxides. This makes not only the extraction of DNA and proteins challenging but also prevents the application of sensitive techniques, such as flow cytometry or isotope labeling, which find application in sand filters treating metal-free surface waters [3,4]. Despite these hurdles, attention toward groundwater biofilters continues to increase, particularly on the biological front. Leveraging this growing momentum, we challenge the field by discussing whether a more ecologically informed control over the microbiome is the key to designing, operating, and controlling the next generation of groundwater biofilters. To do so, we highlight the current bottlenecks, present the latest innovations in this and adjacent fields and, most importantly, critically assess whether ongoing research aligns with the practical needs for drinking water production. We identify nine key focus areas and research questions (RQs).

This *Current Opinion* focuses on the four main ionic groundwater contaminants, namely, iron, ammonia, manganese, and arsenic. We excluded gaseous

Figure 1



Biological and physicochemical reactions involved in the transformation of the main groundwater contaminants, namely, iron, arsenic, manganese, and nitrogen. Oxidation processes are divided into chemical (homogeneous, surface-catalytic) and biological. Homogeneous reactions take place when both reactants are in the same phase, in this case, the water phase, and are especially relevant for iron (with O_2) and As (with ROS that are generated from iron oxidation and, to a lesser extent, with O_2). Surface-catalytic oxidation is relevant for iron (catalyzed by Fe oxides), manganese (commonly known as autocatalytic, by Mn oxides), and arsenic (by Mn oxides). Biological reactions are driven by microorganisms. Nitrogen transformations are exclusively biological, either as the canonical two-step nitrification carried out by AOB and NOB or as the one-step comammox. The (by)products of all these simultaneous reactions interact with each other, and these interactions play a critical role in determining the fate of each contaminant. Our current understanding is that ROS produced during homogeneous iron oxidation oxidize As(III), while iron flocs and iron oxides adsorb As(V) and As(III). Iron flocs inhibit nitrifying bacteria, and Fe(II) oxidizes to Fe(III), while Mn oxides are reduced. Mn oxides are also reduced by nitrite, which is oxidized to nitrate. All the depicted reactions are feasible and proved to occur in sand filters. The inhibition of nitrifying bacteria by iron flocs and the chemical oxidation of nitrite couple of reduction of Mn oxides are the only exceptions still awaiting experimental confirmation in full-scale filters. Abbreviations: MnOx, manganese oxides; FeOx, iron oxides; ROS, reactive oxygen species; AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea; comammox, complete ammonia-oxidizing (bacteria); NOB, nitrite-oxidizing bacteria.

compounds commonly present in groundwater, such as hydrogen sulfide and methane, as they are currently most often stripped during pre-aeration. However, methane-oxidizing bacteria are commonly found in sand filters [5], and the interest in biological methane removal to decrease the greenhouse emissions from drinking water production is increasing [5]. Future opinion papers on sand filtration may consider including methane as the fifth main groundwater contaminant.

Iron: harnessing the wealth of information on iron-oxidizing bacteria

Iron is always the first dissolved contaminant to be removed, regardless of process conditions. Three different mechanisms contribute to iron removal [6] (Figure 1, red section). Homogeneous (flocculent) oxidation is the chemical reaction between dissolved iron (Fe(II)) and dissolved oxygen, resulting in the production of iron flocs (Fe(III)). Surface-catalytic (adsorptive) oxidation is the two-step chemical reaction where dissolved iron binds to

an iron oxide on the surface of a filter-medium grain and is subsequently oxidized to Fe(III) [6]. The product is a compact iron oxide that becomes part of the grain coating. Finally, Fe(II) can be oxidized by iron-oxidizing bacteria (FeOB), yielding relatively compact iron oxide solids as well [7]. Biofilters were, and still are, designed to promote flocculent iron oxidation, a fast, simple, and effective method. However, iron flocs are fluffy, loosely bound structures with high water content, making them prone to clog biofilters. Among others, iron floc accumulation forces frequent backwashing [6] and delays the removal of other groundwater contaminants [2].

Muller et al. [7] designed and operated a groundwater biofilter capable of exclusive adsorptive/biological iron removal at low pH and oxygen concentrations. The absence of iron flocs allowed for a threefold higher flow rate, longer runtimes, and a 75% reduction in energy/water losses, showcasing the benefits of the adsorptive/biological route over conventional flocculent iron oxidation. Interestingly, the morphology of filter-medium grain coating was different from those of conventional biofiltration systems, representing a novel FeOB biosignature. Another recent example of biosignature are the as-of-yet unidentified peaks detected by gas chromatography–mass spectrometry, which result from low-molecular-weight molecules unique to all known FeOB [8]. These findings allow for a simple, fast identification of biological iron removal even in the absence of detectable organisms and potentially beyond those forming the classic twisted stalks, hollow sheaths, or dreads [9,10].

Fast, simple methods to identify and characterize FeOB are essential to promote and control biological iron oxidation. Unfortunately, FeOB have historically been particularly challenging to identify. Yet, the recent rapid advance of the field has made the task considerably easier. First, information about FeOB phylogenetic distribution has always been scarce, hindering their taxonomic identification. The pangenomic analysis of the *Zetaproteobacteria* class [9] and *Gallionellaceae* family [11], encompassing most known iron oxidizers, has resolved the FeOB phylogeny. Second, iron oxidation genes had only been identified as *candidates* in neutrophilic organisms. Moreover, the available information was limited to iron oxidases, while the other genes involved in the pathway were unknown. As a result, FeOB identification via marker genes has also been challenging. Now, the iron oxidation function of cytochrome Cyc2, the most commonly found iron oxidase, has been experimentally validated [12]. Its presence, along with other iron oxidases and additional genes of the iron oxidation pathway, has been detected at metagenomic [13,14], metaproteomic [2], and metatranscriptomic [15,16] level in both natural and engineered settings.

Moreover, candidate genes for other functions in the iron metabolism, such as electron transfer [17] and twisted stalk formation [18], have been recently proposed, and iron genome mining is now possible thanks to FeGenie, an annotation tool for iron-related genes in (meta)genomes [19]. Leveraging exclusive adsorptive/biological iron oxidation is crucial to bring groundwater biofiltration to the next level, and the recent blossom of information about FeOB is an essential first step. Ahead of us lies the next challenge:

RQ1: How do we translate the newly generated knowledge on biological iron oxidation into guidelines to design the next generation of groundwater biofilters?

Ammonia: bridging the gap between academia and practice

The nitrogen cycle is pivotal in bioremediation, for example during wastewater treatment, and as such widely studied. However, the unique conditions in groundwater treatment for drinking water production, that is, low temperatures and oligotrophy, make direct translation of this vast knowledge challenging. The drinking water biofiltration field focused on unraveling the taxonomy of the core nitrification microbiome. While in constant evolution [20], the current understanding is that ammonia oxidation is carried out primarily by ammonia-oxidizing and comammox bacteria [21], with minor contributions from ammonia-oxidizing archaea [22] (Figure 1, green section). Significant efforts are also being devoted to resolving the effect of biofilters operational parameters on microbial community composition and nitrification capacity, such as reported for surface water in terms of backwashing and filter-medium type [23], temperature [24], and flow configuration [25]. Yet, the newly generated insights did not translate into new filter designs or performance improvements.

Central challenges in groundwater biofiltration remain the fact that nitrification often onsets only after full iron removal and that not all influent ammonium is removed, or nitrification stops at nitrite. Usually tightly connected, these problems have been tackled only recently and nitrification remains unpredictable. Several studies demonstrate that sorption of essential nutrients onto the surface of iron oxides is a common cause for incomplete nitrification [26–28]. Wagner et al. [26] proved this principle for copper and achieved a significant increase in nitrification via external copper dosing. The same strategy has also been successfully applied in a full-scale biofilter treating anoxic groundwater [27], and the work of Zheng et al. [28] proved that also phosphate can become limiting. In some cases, nitrification problems are

associated to specific compounds, although the inhibition mechanism is unknown. For example, iron flocs seem to delay the start of nitrification until Fe^{2+} is completely depleted [29]. In other cases, operation fails but the root cause cannot be traced back [30]. These are common observations in practice, yet they receive little to no attention in the scientific literature. To bridge the gap between the focuses of academia and the needs of practice, we identified three crucial areas and RQs that need to be addressed.

RQ2: Reactive oxygen species formed during iron oxidation are known to inhibit ammonia oxidation at nanomolar concentrations [31], dissolved iron has been reported to cause oxidative stress [32], and manganese oxides alter nitrification [33]. What is the direct effect of iron and manganese oxidation (by)products on ammonia-oxidizing organisms, and to which extent does this occur in biofilters?

RQ3: Nitrite, free nitrous acid, and hydroxylamine potentially accumulate during (de)nitrification. These intermediates hamper ammonia oxidation directly [34] and potentially also indirectly by reducing the already formed Fe or Mn oxides and generating products that subsequently interact with ammonia oxidizers. What is the impact of reactive intermediate nitrogen species on key biofilter processes, and what is their implication for process stability?

RQ4: The effect of operational conditions on biofilter microbiome composition is being explored, yet what remains largely unknown is how taxonomic changes reflect in biofilter performance. Does taxonomy matter, and is it a good indicator for process performance?

Manganese: opening the black box

Manganese removal can be of chemical and biological origin (Figure 1, gray section). Surface-catalytic (auto-catalytic) manganese oxidation is a chemical reaction in which dissolved manganese (Mn(II)) adsorbs onto manganese oxide coating on the surface of filter-medium grains. Once adsorbed, manganese is subsequently oxidized to Mn(III) or Mn(IV) and forms a compact oxide that is incorporated into the coating. Biological manganese oxidation is catalyzed by manganese-oxidizing bacteria (MnOB) and results in compact manganese oxides as well. The morphology and properties of biological and chemical oxides differ [35], and references therein), yet both harbor the ability to catalyze surface-catalytic manganese oxidation.

In newly established groundwater biofilters, manganese removal is always the last to meet water quality standards. Consequently, substantial efforts have been put into shortening filter start-up times. The current

understanding is that MnOB initiate manganese oxidation, and chemical reactions dominate in mature filters [36]. A plethora of successful strategies have been developed based on this principle, focusing either on promoting the growth of MnOB [28] or on the addition of mature sand coated with manganese oxides [37]. The biggest challenge for Mn removal remains ensuring long-term stable operation [38]. The complexity stems from the fact that manganese removal takes place in the downstream sections of a biofilter, where filter media are exposed to both residual groundwater contaminants and their (by)products from upstream reactions. High iron [39] loads have been reported to negatively influence manganese removal. Nitrogen species are also expected to impact Mn removal, as observed for ammonia [40] and nitrite [41] in surface water filters. Additionally, oxygen depletion in the low sections of biofilters treating groundwater with high contaminant concentrations may occur. Under anoxic conditions, manganese oxides become the strongest oxidant in the biofilter environment and catalyze the oxidation of Fe(II) , nitrite, and As(III) . Even small fluctuations in the performance of the upper filter sections may induce the downstream reduction of manganese oxides back to dissolved Mn(II) [42•] and ultimately manganese release in the effluent. The emerging oxidation–reduction cycles cannot be fully explained with currently available snapshot-based studies. In the pursuit of long-term, stable manganese removal, two main questions are identified.

RQ5: Which groundwater components or reaction (by)products impact manganese (a)biotic removal the most?

RQ6: What are the underlying mechanisms controlling manganese oxidation–reduction cycles in biofilters, and can they be prevented?

Recent studies challenge also the rationale of MnOB role being limited to the filter start-up phase [39]. The lack of consensus originates from the inherent challenge of identifying the organisms responsible for manganese oxidation. The ability to oxidize manganese is taxonomically more widespread than iron and ammonium oxidation, requiring sequencing at strain-level resolution, and is not necessarily associated with energy conservation [43]. Several enzymes have been proven to catalyze manganese oxidation, with multicopper oxidases being the most common. However, the known substrate promiscuity of multicopper oxidases hinders their use as marker gene to univocally infer manganese oxidation potential in complex communities [44]. Elucidating the *in situ* contribution of biology to manganese removal during groundwater filtration remains an extremely challenging task. Deepening our understanding of manganese-oxidizing organisms and developing fast analytical methods for their rapid identification is crucial

to control and optimize manganese removal in biofilters. The central open fronts are summarized in one question.

RQ7: To what extent is Mn biologically removed in biofilters, and what are the responsible organisms?

Arsenic: promoting removal through biological oxidation

Arsenic removal in biofilters primarily relies on adsorption to iron oxides. In anaerobic groundwater, the oxyanion arsenite (As(III), H_3AsO_3) is the dominant arsenic species. However, iron oxides have a higher adsorption affinity for its oxidized, negatively charged counterpart arsenate (As(V), H_2AsO_4^- , HAsO_4^{2-}) [45]. This emphasizes the importance of As(III) oxidation to As(V) to ensure efficient removal. Since homogeneous As(III) oxidation with dissolved oxygen is slow (days), surface-catalytic As(III) oxidation by Mn oxides [46] and homogeneous oxidation by reactive oxygen species [47] have long been claimed to be the dominant As(III)-oxidizing mechanisms (Figure 1, yellow section). Yet, several researchers have indicated that microorganisms also play a role in arsenic oxidation [48–51], achieving a higher oxidation rate than the abiotic process alone [52]. However, experimental proof in sand filters is missing.

Biological As(III) oxidation is carried out by chemolithoautotrophic organisms for energy conservation as well as by heterotrophs as detoxification strategy using oxygen or nitrate as electron acceptor [53]. Arsenic-oxidizing bacteria (AsOB) have been widely detected in aquifers [50] and lab-scale biofilters [48,49] either by PCR (polymeric chain reaction)-based methods targeting As(III) oxidase *aox* or with metagenomic-based approaches [51,54•]. However, both methods share a major limitation that compromises the reliable detection of AsOB. The low concentration of AsOB in biofilters resulting from micromolar arsenic concentrations in groundwater (2–3 orders of magnitude below ammonia) and the suboptimal DNA extraction yields from metal-coated sand grains make AsOB identification challenging, often practically impossible. Ultimately, to develop filter designs targeting also arsenic removal, a paramount requirement in areas where arsenic exposure via drinking water remains a major health risk, we identified two areas and questions of utmost interest.

RQ8: The lack of established methods to detect AsOB and differentiate between biological and chemical As oxidation processes prevents further understanding of the dominant As oxidation mechanisms in biofilters, let alone identifying which one should be promoted to improve filter performance. How could biological As oxidation and chemical oxidation with Mn oxides be differentiated?

RQ9: While As uptake by abiotically formed Fe oxides is a well-known process, the as-of-yet answered question is: what is the uptake of arsenic during biological Fe oxidation?

Perspective

Groundwater biofiltration research is rapidly growing. Complex and interdisciplinary challenges are being solved, and our vision of biofilters is evolving from *black* toward *white box*. Besides the long established knowledge on the underlying physicochemical processes, the prominent role of the biofilter microbiome in process robustness is emerging and sets the foundations for new design opportunities. Promoting adsorptive/biological iron removal seems to be a superior strategy compared to the decades-old conventional homogeneous iron oxidation. By preventing loose iron flocs formation, adsorptive/biological iron removal extends filter runtime and prevents the potential inhibition of the downstream ammonia and manganese removal. The importance of iron oxidation (by)products on ammonia-oxidizing organisms is also being recognized, and more research is needed to understand the causes of often reported nitrification failures. The discharge of nitrification intermediates, such as nitrite, impacts water quality both directly and indirectly, as it may promote the largely overlooked reduction and redissolution of previously precipitated manganese. The contribution of biotic reactions to manganese removal remains to date unclear, and we encourage the scientific community to concentrate efforts to gain a deeper understanding of the differences in filter performance and (by)products of biological and chemical manganese oxidation. In contrast, the paradigm is changing for arsenic, and evidence is growing for biological oxidation to be its dominant removal mechanism. Efforts should focus on confirming the biotic nature of arsenic removal and on optimizing filter design to promote the growth of arsenic-oxidizing bacteria. Within this framework, metagenomic and metaproteomic approaches provide unprecedented possibilities to resolve the microbial metabolic network in biofilters. Research should target the optimization of protocols to extract DNA and proteins from a biomass enmeshed with solid precipitates. The rapid progress in understanding the controls of biofilter microbiomes undoubtedly provides a new operational dimension to design and operate the next generation of groundwater biofilters. However, scientific insights into removal mechanisms remain detached from operational practice, hindering the implementation of the new understandings to improve filter performance. Combining the efforts of microbiologists, biotechnologists and bioprocess engineers with applied research is now critical to bridge the gap between individual removal mechanisms and full-scale sand filters complexity.

CRedit authorship contribution statement

Francesc Corbera-Rubio: Conceptualization, Investigation, Writing – original draft. **Roos Goedhart:** Conceptualization, Investigation, Writing – original draft. **Michele Laurenzi:** Conceptualization, Writing – review & editing, Supervision. **Mark van Loosdrecht:** Conceptualization, Writing – review & editing, Supervision. **Doris van Halem:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Data Availability

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

Declaration of Competing Interest

The authors declare no conflict of interest.

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