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# Applying biotechnology for drinking water biofiltration: advancing science and practice

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Drinking water biofiltration processes have evolved over time, moving from unintentional to deliberate, with careful filter media selection, nutrient and trace metal supplementation, oxidant amendment, and bioaugmentation of key microorganisms, to achieve improvements in water quality. Biofiltration is on the precipice of a revolution that aims to customize the microbial community for targeted functional outcomes. These outcomes might be to enhance or introduce target functional activity for contaminant removal, to avoid hydraulic challenges, or to shape beneficially the downstream microbial community. Moving from the foundational molecular techniques that are commonly applied to biofiltration processes, such as amplicon sequencing and quantitative, real-time polymerase chain reaction, the biofiltration revolution will be facilitated by modern biotechnological tools, including metagenomics, metatranscriptomics, and metaproteomics. The application of such tools will provide a rich knowledge base of microbial community structure/function data under various water quality and operational conditions, where this information will be utilized to select biofilter conditions that promote the enrichment and maintenance of microorganisms with the desired functions.

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## Introduction

Biological water treatment processes rely partially or entirely on biological mechanisms to achieve treatment objectives. These processes broadly include natural (e.g., riverbank and aquifer filtration) and engineered (e.g., fluidized bed as well as slow sand and rapid-rate filtration) processes. In drinking water treatment, 'biofiltration' processes in North America typically involve rapid-rate, granular media filters that are similar in design to conventional, physico-chemical filtration processes [1–3], whereas slow sand filters see continued use internationally. Biofilters differ from conventional filters through key operational practices that promote and maintain biological activity on the filter media, which enhances the transformation of organic and inorganic constituents before treated water is introduced into the distribution system (American Water Works Association Biological Drinking Water Treatment Committee, J. Carter, personal communication). In this *Current Opinion*, we draw heavily on the literature from the past three years to review advancements in the science and practice of drinking water biofiltration as well as to discuss the potential role of modern biotechnological tools to further this advancement.

## The state of drinking water biofiltration

In contrast to conventional, physico-chemical filtration, biofiltration is used to reduce the biodegradable fraction of dissolved natural organic matter (NOM). NOM removal is a common driver for biofiltration in North America [3] with the following potential benefits: improved biostability in the distribution system, removal of contaminants of emerging concern [4] and taste and odor compounds, reduced membrane fouling [2], and, in systems that include disinfection and/or post-filter disinfection, removal of disinfection by-product precursors [5] and reduced chlorine demand [2]. NOM removal by biofiltration has been extensively reviewed (e.g., Refs. [6•,7]). Likewise, biofiltration is an important process for the transformation of inorganic contaminants, particularly in groundwater systems. For example, biofiltration has been studied for the removal of ammonia [8•], arsenic [9], bromate [71], iron [10], manganese [10], nitrite [11], and perchlorate [12].

Historically, 'classical' biofiltration has been the most common type of drinking water biofiltration [3]. It occurs when conventional filtration processes, ranging from rapid-rate to slow sand filters, are operated in the absence of

chlorine in the filter influent. Upstream oxidation with ozone, ultraviolet light (UV)/hydrogen peroxide, or UV/ozone generally increases the biodegradability of NOM, increasing NOM removal by biofiltration. As the treatment performance and cost benefits of biofiltration have been increasingly recognized, drinking water biofiltration research has shifted from the assessment of process performance to the development of design and operational strategies (i.e., media type and configuration, contact time, hydraulic loading rate, and backwash and pre-treatment strategies) for enhanced treatment [1,2]. More recently, the focus of biofiltration research has shifted again, signaling a recognition that further enhancements in treatment performance will require customizing the microbial communities to achieve targeted objectives. The purposeful tailoring of microbial community structure (i.e., relative abundance of various microorganisms) will require modifications beyond the pre-treatments to increase NOM biodegradability that are typically associated with classical biofilter operation. Supplementation of compounds already present in filter influents (e.g., limiting nutrients such as phosphorus) [13•,14], amendment of compounds absent from filter influents (e.g., oxidants) [15,16], or bioaugmentation with key microorganisms (i.e., those that do not naturally develop in biofilters or those that grow very slowly) [17,18] are strategies that can be considered to enable and/or increase the transformation of target contaminants (Figure 1).

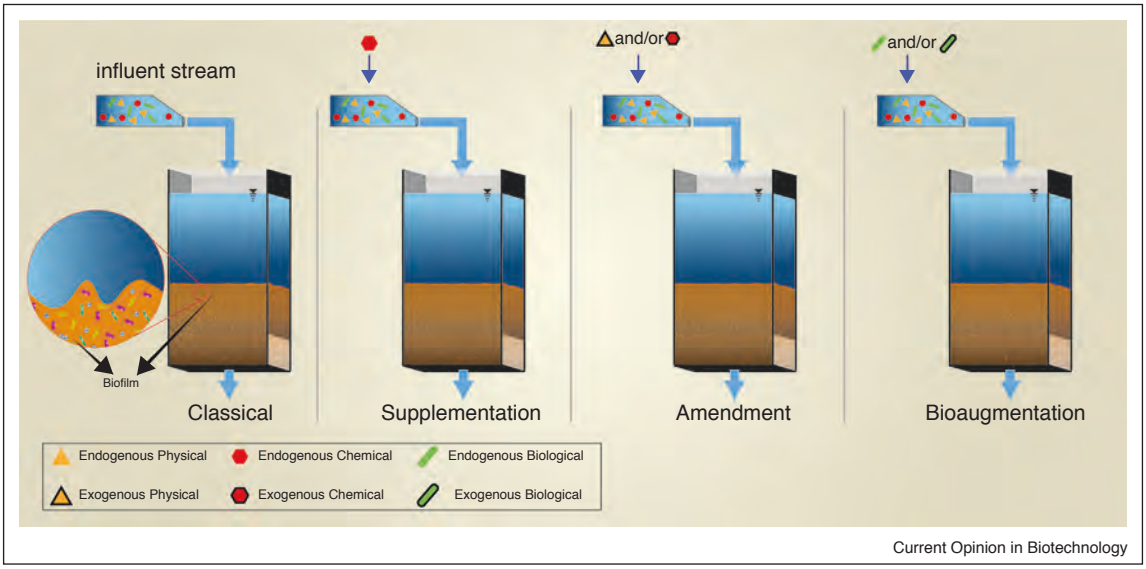
Effective customization of microbial community structure requires a detailed understanding and characterization of the community. Fortuitously, the past decade has delivered unprecedented advances in this domain. As discussed by Zhou *et al.* [19], 16S rRNA gene characterization — though foundational to modern microbial community analysis — has limited quantitative utility [20,21]. Metagenomics overcome this limitation by identifying novel microorganisms and their functional potential [22,23,24•] by exploiting a fully *de novo* approach [25••]. The increase in analytical throughput and commensurate decreases in cost of DNA sequencing make metagenomic approaches increasingly accessible [26].

A new era of drinking water biofiltration has emerged and will be focused necessarily on the mechanistic linkages between microbial community structure and biofilter function. An improved understanding of how the structure, function, and dynamics of microbial communities contribute to biofiltration processes and how they can be customized to improve treatment efficacy are critical to advancing the science and practice of biofiltration.

## Customizing drinking water biofiltration processes

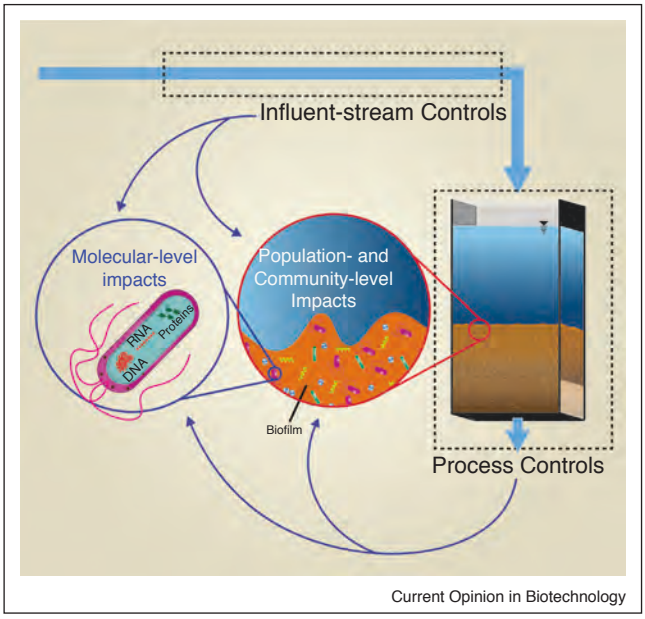
Biofilter control can be exerted at the process (i.e., design and operation) and influent-stream levels (Figure 2) to customize a biofiltration process to achieve a targeted functional outcome.

**Figure 1**




Generalized biofiltration process configurations, including classical, supplementation, amendment, and bioaugmentation. *Classical* biofiltration is often associated with pre-treatments to improve the biodegradability of the NOM. Shapes without a black outline represent water quality components that are naturally present (endogenous) to the filter influent; in biofilter *supplementation*, an endogenous component can be added to reach a higher concentration in the filter influent than is present naturally. Shapes with a black outline represent water quality components that are not naturally present in the filter influent (exogenous); addition of an exogenous physical or chemical component is termed *amendment*, and addition of an endogenous or exogenous biological component is termed *bioaugmentation*.

**Figure 2**




Influent-stream and process controls affect biofiltration processes at the population, community, and molecular levels.

At the process level, filter media selection can impact the biofilter at the population and community levels (i.e., which organisms are present) and the molecular level (i.e., the functional potential and activity of those organisms as encoded by DNA, RNA, and proteins). Recently, Spanjers [27] debunked the conventional wisdom that the roughness of granular activated carbon (GAC) protects biofilm from shear forces and leads to improved dissolved organic carbon (DOC) removal relative to smoother media. Spanjers [27] found that the adsorptive nature of GAC, not media roughness, is essential to that improved DOC removal (even over the long-term). Additionally, filter media can impact microbial community structure; recent evidence suggests that GAC biofilter communities tend to be more diverse than those on other filter media types [28]. In particular, Vignola *et al.* [29] found greater phylogenetic diversity in GAC as compared to sand biofilters. Interestingly, they also demonstrated that stochastic factors have a much smaller impact on biofilter community assembly relative to deterministic factors such as filter media type, process operation (e.g., empty bed contact time) or other controllable parameters such as pH, dissolved oxygen concentration, NOM character/concentration, and nutrient concentrations, further highlighting the importance of such parameters.

At the influent-stream level, oxidant amendment and nutrient supplementation can influence process-level properties (e.g., filter hydraulics) and population-level and community-level properties (e.g., microbial community structure and functional potential/activity). Tailoring the influent concentrations of chlorine, chloramines, or hydrogen peroxide to shift the biologically active zone below the main particle capture zone in a filter is one way to lower headloss accumulation without substantially impacting the removal of NOM and turbidity as compared to biofilters with no oxidant addition [16]. However, the implementation of chlorine or chloramine for these benefits must be weighed against their ability to impact microbial community structure [30,31] within the biofilter and the subsequent seeding from the biofilter to the distribution system [32]. Copper limitation can curtail nitrification [33] because it is essential for the activity of the ammonia monooxygenase enzyme [34]; hence, copper supplementation has been shown to rectify incomplete nitrification in biofilters [8•]. When biofilters are truly limited by phosphorus (as demonstrated by the ratio of phosphatase to glycosidase enzyme activities), supplementation of phosphorus decreases the concentration of extracellular polymeric substances (EPS) on the filter media and the rate of headloss accumulation [13•]. The stringency of phosphorus limitation also has a substantial impact on microbial community structure [13•].

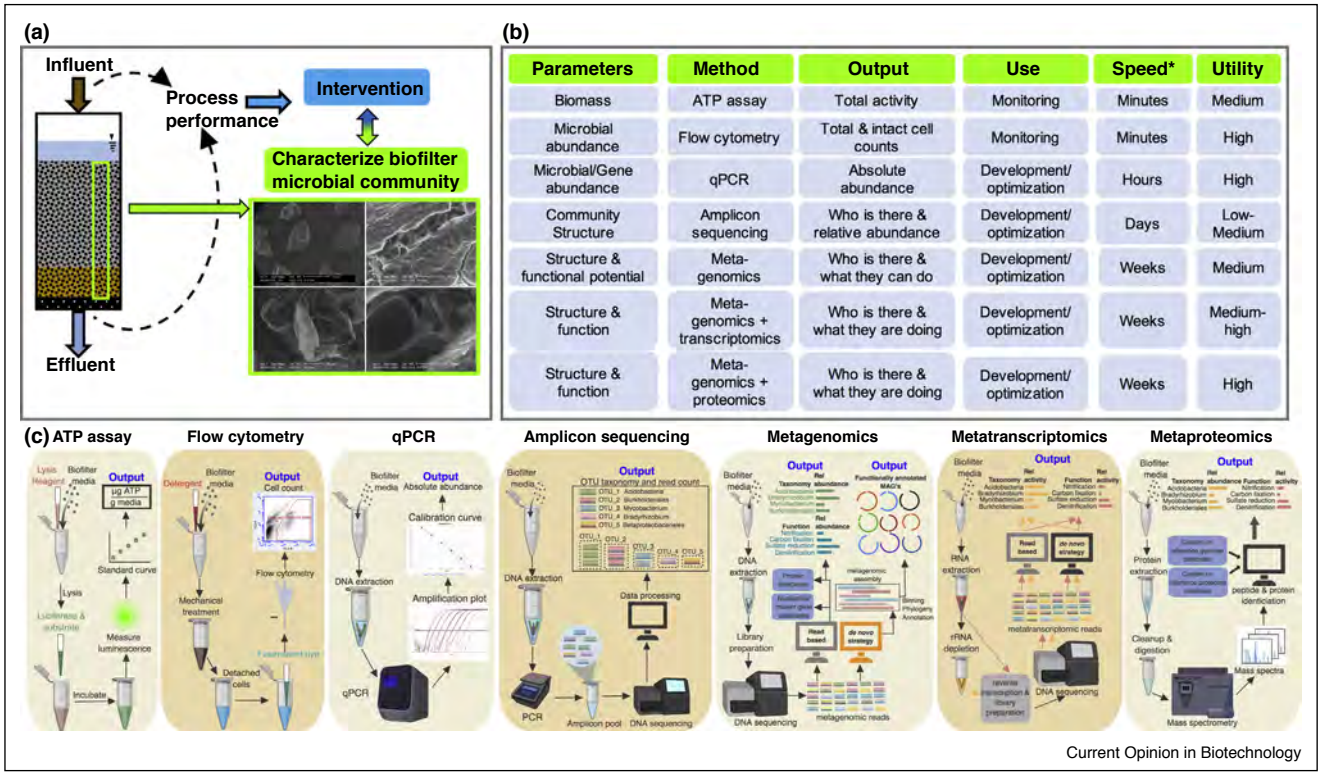
Bioaugmentation of key microorganisms can be effected at the influent-stream level or directly within the process by incorporation of existing biofiltration media, with subsequent population-level, community-level, and molecular-level impacts. Albers *et al.* [17] found that bioaugmenting a fresh filter with a nitrifying consortium from an existing biofilter enriched on quartz sand substantially decreased the lag time before nitrification commenced in the new filter. However, the bioaugmented microorganisms were eventually outcompeted by native nitrifiers, similar to the long-term loss of bioaugmented microorganisms observed in other drinking water studies [71]. However, immobilizing bioaugmented microorganisms into fixed carriers can prolong the efficacy of the bioaugmentation strategy in a short-term manner [18].

## Biotechnological methods to advance the science and practice of biofiltration

As summarized in Figure 3, molecular methods can be utilized for biofilter (i) monitoring and (ii) improvement. Selective monitoring of biofilters with molecular methods will facilitate the development of a rich knowledge base that provides links between microbial community structure/function and treatment performance, such as pollutant removal. Utilizing this knowledge base, improved biofiltration treatment performance could be obtained via targeted 'interventions' (e.g., pH adjustment or nutrient supplementation) to manipulate the microbial community structure and function.

Each molecular method's utility depends on its turnaround time and the type of information it provides. For instance, biomass monitoring using adenosine triphosphate (ATP)

**Figure 3**




**(a)** Microbial community characterization combined with process performance can be used to design process interventions to manage the microbial communities of biofilters. **(b)** An overview of methods used for biofilter monitoring and improvement, their output type, and utility. The qualitative speeds (i.e., sample-to-data turnaround times) associated with each method indicated in the figure assume in-house sample processing, no queue time, and optimized, ready-to-use data analyses workflows. **(c)** Overview of the workflow and key outputs from each method identified in panel (b).

assays is widespread due to its fast turnaround time and availability of easy-to-use commercial kits [36]. Relatedly, flow cytometry for total and intact cell counts (i.e., those with intact cytoplasmic membranes) can be performed continuously and in near real-time [35]. While widely applied to bulk water, recent protocol developments indicate that cell counting for biofilter media is feasible [37,38]. Further, combining fluorescence and scatter data from flow cytometry analyses provides a powerful microbial community fingerprint, referred to as 'phenotypic diversity' by Props *et al.* [39••]. The phenotype-resolving capacity of this approach is limited, but changes in phenotypic diversity correlate with changes in microbial community structure as estimated by DNA sequencing [40]. Both ATP and flow cytometry are well suited for biofilter monitoring, but they are of limited utility for biofilter improvement because they do not provide information about microbial community structure and function or their link to biofilter performance.

Dissecting microbial community structure and function requires interrogation of DNA, RNA, or proteins. Quantitative, real-time polymerase chain reaction (qPCR) analysis has been used to quantify genes encoding enzymes that catalyze functions of interest in biofilter microbial communities [9,41]. While quantitative, the requirement for prior knowledge of the target gene sequences to enable robust primer design is an important challenge. In contrast, highly multiplexed amplicon sequencing that targets hypervariable regions of the small subunit (SSU) rRNA gene [42] can be used for comprehensive microbial community analyses to assess operational impacts on biofilter microbial communities [28]. Amplicon sequencing data are usually processed to the level of operational taxonomic units (OTUs) [43] or amplicon sequence variants (ASV) [44] as biologically relevant units of measurement. OTU/ASV sequences are assigned taxonomy (typically to the order, family, or genus level) by utilizing public databases [45–47], and their raw, subsampled, or normalized read counts are employed to characterize microbial community membership (i.e., who is present) and structure [48,49]. While relatively inexpensive, amplicon sequencing is (i) not quantitative [20,21], (ii) has limited phylogenetic resolution [50], and (iii) cannot be used to infer OTU/ASV function [51]. In contrast, shotgun DNA sequencing (i.e.,

metagenomics) [25••,52] can provide insights into microbial community membership, structure, and functional potential. Metagenomic read profilers provide a database-dependent catalog of community taxonomy [53–55] and functional potential but are unsuitable for *linking* structure with functional potential [56•]. Doing this requires a *de novo* approach involving metagenomic assembly and binning to obtain metagenome-assembled genomes (MAGs) [52,57]. This results in clustering of phylogenetic markers and functional genes into individual population MAGs [23,24•]. Further, metagenomic analyses can provide absolute abundances of detected populations and MAGs through the use of internal standards spiked into the extracted DNA before library preparation and sequencing [58]. Though phylogenetic placement of novel MAGs is possible, identifying novel functions is non-trivial [59], and, thus, MAG functional annotation is entirely database dependent. While metagenomics does not provide proof of microbial activity and contribution to biofilter performance, this limitation can be overcome partially by complementing metagenomics with RNA-based analyses, such as by mapping metatranscriptomic reads to MAGs to determine if the targeted functions are being expressed (and by which microorganisms).

Microbial community structure and function can be linked with biofilter performance by coupling stable isotope probing with sequencing approaches to identify microorganisms that assimilate labelled contaminants [60–62]. However, this is not useful for pollutants removed via dissimilatory or co-metabolic mechanisms. Likely, the most powerful approach for this purpose is metaproteomics [63]. Mass spectrometry-identified peptide signatures coupled with custom proteome databases (e.g., proteome predicted from metagenome) can be used to identify and count proteins, which can shed light on microbial presence, activity, and function [64,65••]. Further, multiplexing and quantitative metaproteomics also are feasible by combining isobaric labelling with estimation of total extracted protein concentration, respectively [64]. Compared to the other methods detailed above, protein presence/absence and abundance variation in response to biofilter operation, pollutant concentrations, and/or water quality conditions would be the most useful data for informing process strategies to improve biofilter performance because these measurements demonstrate if the biofilter community is actively expressing proteins capable of catalyzing the target biotransformation. However, it must be noted that the ability to annotate proteomic data is dependent on the depth and breadth of protein databases, where proteins involved in unknown or poorly characterized biotransformation pathways are unlikely to be annotated.

The DNA-based, RNA-based, and protein-based methods outlined above are best suited for selective monitoring and biofilter improvement. They are unsuitable for routine monitoring on a daily or weekly basis due to complex sample and data processing requirements. Nonetheless, recent developments in nanopore platform-based DNA [66] and RNA [67] sequencing and protein profiling [68] might usher in a new era of high-resolution, real-time, structure-function monitoring for biofilter microbial communities.

## Conclusions and future perspectives

Drinking water biofiltration is on the precipice of a revolution that aims to tailor the microbial community to the desired functional outcome of the process. Building on the successes of 16S rRNA gene sequencing to determine 'who' populates biofilters and of qPCR to quantify functional genes of interest, the drinking water community is poised to execute this revolution utilizing a plethora of modern biotechnological tools. These tools will inform process strategies to shape biofilter communities at the community, population, and molecular levels.

To glimpse the possibilities availed by biofilter customization, one needs to look no further than the explosion of studies to manipulate the human gut microbiome for a beneficial functional outcome. For instance, the MyNewGut project aims to reduce the risk of human disease by utilizing dietary interventions that directly affect the gut microbiome [69]. Just as the human gut microbiome is thought to impact the quality of human health on many levels, so too are biofiltration processes a key way to influence human health. Using the same cutting-edge biotechnological tools that are employed in human microbiome research, we can develop customized biofiltration processes to produce safe water; furthermore, in a conducive regulatory environment, we might also utilize biofiltration to deliver beneficial microorganisms to consumers [32,70].

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## Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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