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Engineering of managed aquifer recharge systems to optimize biotransformation of trace organic chemicals

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Abstract

Managed aquifer recharge (MAR) systems provide effective removal of many water contaminants including suspended solids, organic matter, pathogens, and numerous trace organic chemicals (TOrCs). TOrC removal is primarily driven by biotransformations performed by subsurface microbial communities. However, variable extents of TOrC biotransformation have been reported across MAR systems. This review discusses major parameters affecting the biotransformation of TOrCs and summarizes recent efforts to enhance its efficiency in MAR systems. Approaches to enhance biotransformation of TOrCs during MAR include optimization of environmental conditions (redox conditions, substrate availability), inoculation of specific TOrC degraders and stimulation of degrader activity by providing growth substrates or co-factors. While concepts to optimize environmental conditions have been tested at different scale, inoculation and biostimulation approaches were mostly tested as a means to remove contaminants in biologically active sand filters or for the remediation of contaminated groundwater. Their application in MAR systems needs further research.

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Biostimulation, Biotransformation, Managed aquifer recharge, Sequential managed aquifer recharge, Trace organic chemicals.

Introduction

The influent of municipal wastewater treatment plants (WWTPs) contains a complex mixture of pharmaceuticals, personal care products, household chemicals, and other down-the-drain chemicals that are collectively known as trace organic chemicals (TOrCs) [1]. Wastewater microbial communities can biotransform many TOrCs to some degree, but many other TOrCs persist during biological wastewater treatment [2]. Consequently, the effluent of municipal WWTPs contains a complex mixture of TOrCs and their biotransformation products [3]. The occurrence of these chemicals in downstream surface water systems has been linked with adverse effects within aquatic ecosystems [4] and can compromise the production of safe drinking water.

In recent years, municipal WWTPs have adopted additional physical (*e.g.*, activated carbon adsorption) and chemical (*e.g.*, ozonation) treatment processes as a means to more completely remove TOrCs from wastewater prior to discharge to the aquatic environment [5,6]. Although physical and chemical treatment processes can be effective at removing many TOrCs from water, upgrading WWTPs to include these advanced treatment processes is generating additional costs and energy demand [7]. Multiple studies have also demonstrated that many types of TOrCs can also be removed during managed aquifer recharge (MAR) [8] which is the intentional recharge of water (*e.g.*, municipal WWTP effluent, surface water, stormwater) to aquifers for the purposes of water treatment and/or storage [9]. As water percolates through the unsaturated and saturated zones during infiltration or after direct injection into the aquifer, TOrCs can be removed by means of adsorption to aquifer media or biotransformed by subsurface microbial communities [8]. The contribution of these mechanisms to the mitigation of individual TOrCs in MAR systems depends on several factors including aquifer characteristics, environmental conditions, and physico-chemical properties of the respective substance. This review is focused on the biotransformation of TOrCs, which is considered to be a sustainable process that can potentially result in TOrC mineralization (*e.g.* Ref. [8]).

At the most fundamental level, the biotransformation of TOrCs during MAR depends on the chemical structure of an individual TOrC and the presence of an enzyme catalyst to initiate the biotransformation [10]. The chemical structure of the TOrC can influence its partitioning behavior in the aquifer and its bioavailability (*e.g.*, speciation, presence of labile functional groups, *etc.*) [11]. The presence of a relevant enzyme catalyst is dependent on the composition of the subsurface microbial community which is determined by the prevailing environmental conditions, such as the redox conditions, temperature, pH, and presence or absence of co-substrates and other nutrients [12]. Other physiological factors including mass transfer limitations (*i.e.*, chemical uptake into the cell) and growth-linked versus co-metabolic biotransformations are also relevant because TOrCs are often present at concentrations that are several orders of magnitude lower than the bulk organic carbon content. To optimize the performance of aquifer microbial communities for the biotransformation of TOrCs in MAR systems, we need a better understanding of the mechanisms by which certain TOrCs are biotransformed and how the environmental conditions shape the composition and the physiology of the aquifer microbial community. Improved insights in these areas could lead to strategies that enhance the biotransformation of TOrCs in MAR systems.

In this review, we will discuss the current knowledge on the underlying principles and mechanisms for the biotransformation of TOrCs in MAR systems and review new ideas and concepts for optimizing the performance of subsurface microbial communities in MAR systems for the biotransformation of TOrCs. We specifically address the optimization of environmental conditions, the inoculation of specific microbial degraders or enzymes (*i.e.*, bioaugmentation), and the stimulation of specific microbial physiologies (*i.e.*, biostimulation).

Biotransformation of TOrCs in MAR systems

Optimal physicochemical conditions for biotransformation of TOrCs

Several studies have addressed the biotransformation of TOrCs in batch-, column- and field-scale experiments representing MAR systems characterized by different environmental conditions [13]. As expected, physicochemical conditions such as the redox conditions, temperature, pH, and presence or absence of co-substrates and other nutrients influence the biotransformation of TOrCs in these experiments [11, 14-16]. Whereas the temperature and pH are determined by the site-specific conditions and are not easy to modify given practical considerations, the redox conditions and the presence or absence of co-substrates and other nutrients could potentially be manipulated in MAR systems for the purposes of optimizing the biotransformation of TOrCs and warrant more careful evaluation.

Predominant redox conditions are a major driver for the biotransformation of individual TOrCs in MAR systems. Anoxic or anaerobic conditions in MAR systems can lead to the biotransformation of some otherwise recalcitrant TOrCs including halogenated chemicals (*e.g.*, iodinated contrast media) or the antibiotic sulfamethoxazole [17,18]. However, most TOrCs are more efficiently biotransformed under oxic conditions [17,16,19], although it is not always clear whether aerobic biotransformations result in mineralization or the formation of persistent biotransformation products [20]. These studies suggest that oxic redox conditions should be maintained to facilitate a more comprehensive biotransformation of TOrCs in MAR systems.

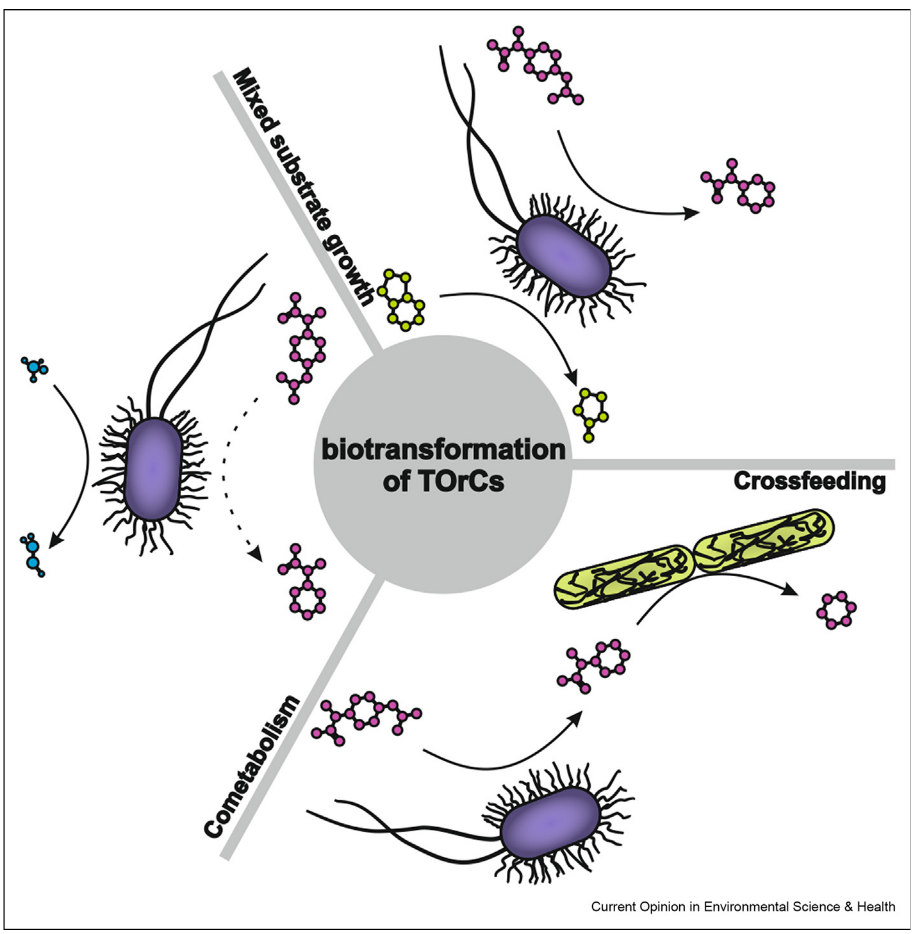
The presence or absence of co-substrates and other nutrients is another major driver for the biotransformation of individual TOrCs in MAR systems. The biotransformation of some TOrCs is positively associated with the abundance of dissolved organic carbon (DOC) and dissolved oxygen in both laboratory-scale systems and in field studies [13,21], demonstrating greater extents of biotransformation in systems with greater microbial activity. However, in some cases the available organic matter can also adversely affect the attenuation of TOrCs as it was observed for algal organic matter [22]. In general, the biotransformation of some TOrCs is favored only in carbon- or nutrient-limited systems [12]. Carbon-limited systems support the growth of more taxonomically and functionally rich microbial communities [23] which can have a positive effect on the biotransformation of otherwise recalcitrant TOrCs [24,25]. These findings were also corroborated in a recent study that examined compound-specific removal patterns for a diverse group of TOrCs in controlled column experiments [26]. Whereas many TOrCs were rapidly biotransformed in columns with a high abundance of DOC, the biotransformation of others was only effective under carbon-limited conditions. A comparison of initial reactions reported in the literature suggested that microbial communities under carbon-limited conditions utilize different enzymes to initiate the biotransformation of TOrCs [26]. These studies suggest that a combination of different substrate conditions might lead to a more comprehensive biotransformation of TOrCs in MAR systems.

It should be noted that conclusions on the effect of physicochemical parameters are mostly derived from column studies under controlled conditions. Aquifers in full-scale MAR systems are typically characterized by highly heterogeneous conditions leading to tortuous flow with variable hydraulic retention times and redox conditions [27]. Also, spatially variable sorption and desorption behavior of contaminants may affect the growth of microbial communities in heterogeneous environments. Especially in MAR systems with surface infiltration (*e.g.*, induced bank filtration, soil-aquifer treatment), microorganisms are also shaped by seasonal variations of temperature, flow rates, and redox conditions [28]. System heterogeneity is a challenge for systematic evaluation of TOrC biotransformation in full-scale MAR systems. Some studies utilized statistical data evaluation from different MAR sites to elucidate how redox conditions and hydraulic retention times affect TOrC removal [13,29]. Results from parallel monitoring of two infiltration basins operated with different feed waters indicate that temporal changes of redox conditions can adversely affect biotransformation of TOrCs [30], but to the best of our knowledge systematic studies that address the effect of physical subsurface heterogeneity on biotransformation of TOrCs are limited.

Physiological mechanisms of TOrC biotransformation in MAR systems

The biotransformation of TOrCs in any environmental system (including in MAR systems) is unique in that TOrCs are often present at concentrations that are several orders of magnitude lower than the bulk organic carbon content. The prevailing paradigm on the physiological mechanism of TOrC biotransformation is that environmental concentrations of TOrCs are too low to support the growth of a microbial community. Under this paradigm, biotransformation of TOrCs would be catalyzed by promiscuous enzymes in co-metabolic processes, during mixed substrate growth of microorganisms with a diverse enzyme pool, or through cross-feeding by a diverse microbial community (Figure 1). Evidence for co-metabolism includes an apparent lack of adaptation periods for TOrC biotransformation under carbon-limited conditions [24,31] and associations with the availability or removal of potential co-substrates like ammonia or humic acids [32,33]. However, other studies found no association between TOrC biotransformation and the utilization of refractory humic substances [34], showed that direct effects of ammonia-oxidizing bacteria are limited to a few TOrCs [35,36], and have reported microbial adaptation to individual substances at concentrations less than 1 μg/L [37,38]. These latter results suggest that biotransformation of TOrCs might also involve metabolic processes. A potential strategy is the co-utilization of different substrates or so-called

Figure 1



Different physiological mechanisms that can be involved in biotransformation of TOrCs.

mixed substrate growth [39]. Under such conditions, diverse and promiscuous enzymes readily interact with a variety of potential substrates in growth-linked reactions. Another concept is the symbiotic growth of different microorganisms by cross-feeding, i.e., the utilization of products and intermediates from each other's metabolism. These concepts match the ecological theory on energy tradeoffs for generalists (mixed TOrCs use) versus specialists (individual TOrC use) when TOrCs are considered as substrates. In reality, complex mixtures of TOrCs are likely biotransformed under a continuum of physiological mechanisms that range between co-metabolic processes, mixed substrate growth, cross-feeding, and other growth-linked processes.

Standard methods commonly applied in engineering or microbiology are not appropriate to elucidate physiological mechanisms of TOrC biotransformations in MAR systems with mixed bacterial communities. The biotransformation of low concentrations of TOrCs with a massive level of chemical diversity likely involves a variety of different, potentially interdependent microorganisms, which limits the adaptation of research concepts established for the biotransformation of environmental contaminants that are present at higher concentrations (*e.g.*, isolation of single degrader strains). A recent perspective paper [40] outlined new concepts that could be useful for identifying the taxa and enzymes involved in TOrC biotransformation, determining biotransformation pathways for individual TOrCs, and understanding and predicting microbial transformation of TOrCs in the environment. For example, one suggested approach explores correlations between metagenomics or metatranscriptomics datasets and the rates or extent of TOrC biotransformation across gradients of environmental conditions [41]. Positive and significant correlations that are identified in this way could be further evaluated with additional knowledge on initial biotransformations to improve the identification of key functional genes or transcripts [26,42] and to limit the rate of false positives [41]. Other potential approaches include developing model microbial communities with a smaller number of taxa that can represent complex interactions in natural systems [43,44] or metabolic modeling based on (thermodynamic) flux balance analysis [45]. To the best of our knowledge, these concepts have not yet been adapted to investigate biotransformation of TOrCs.

Optimizing the biotransformation of TOrCs in MAR systems

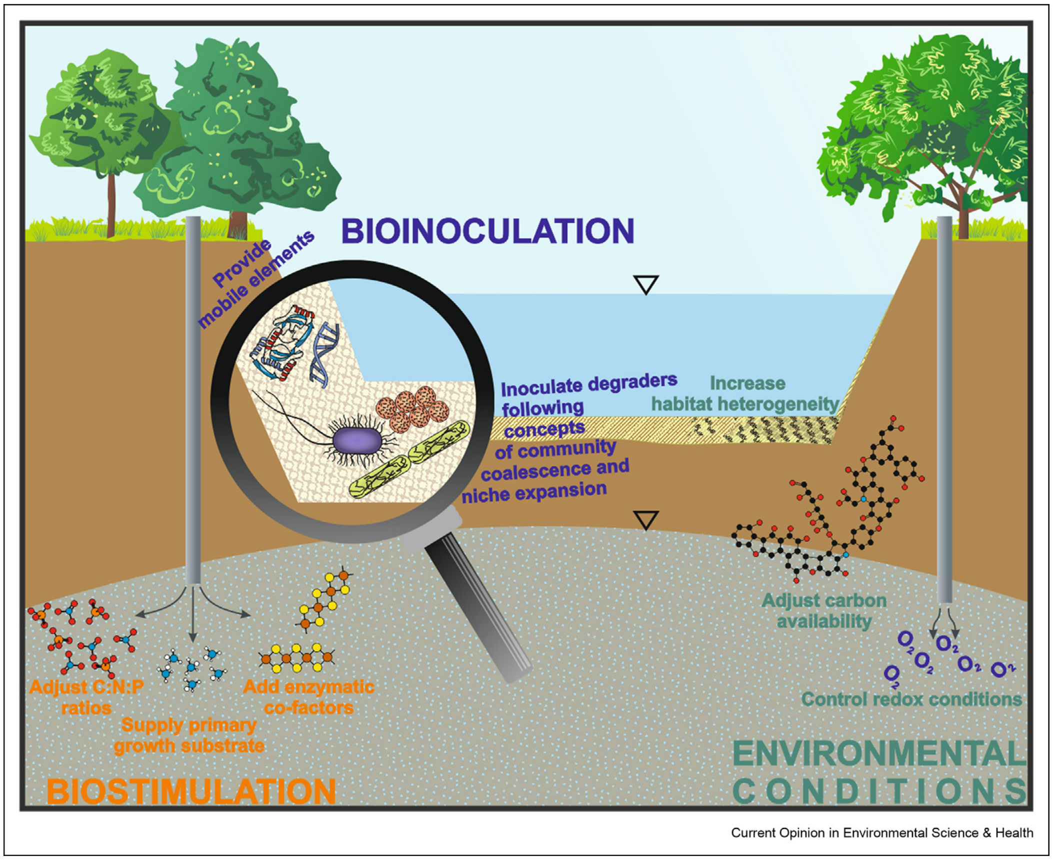
Optimization of environmental conditions for biotransformation

One strategy to optimize the biotransformation of TOrCs in MAR systems is to manipulate the environment to control the redox conditions and/or the presence or absence of co-substrates and other nutrients (Figure 2). Because some TOrCs are known to be biotransformed only under oxic or anoxic to anaerobic conditions and because variable biotransformation of TOrCs has been reported at high and low DOC and nutrient concentrations, one could expect that a MAR system that exploited all of these conditions would exhibit the best performance with respect to comprehensive TOrC biotransformation. Conventional MAR systems that receive municipal WWTP effluent are characterized by a rapid consumption of DOC in the top layer of the infiltration zone corresponding to a rapid depletion of dissolved oxygen [46] and resulting in suboxic and anoxic conditions in deeper layers. Therefore, conventional MAR systems are characterized by an upper layer of carbon-rich and oxic conditions and a lower layer of carbon-limited and anaerobic or anoxic conditions. To obtain the more favorable carbon-limited and oxic conditions, one could incorporate pretreatment to reduce DOC and oxygen demand, or supply additional dissolved oxygen (*e.g.*, through addition of hydrogen peroxide or ozonation) [47].

A new concept that more explicitly exploits controlled redox and co-substrate or nutrient conditions to enhance the biotransformation of TOrCs is the sequential managed aquifer recharge technology (SMART) [48]. In SMART, water from an initial infiltration step (under carbon-limited and anaerobic or anoxic conditions) is pumped to the surface, re-aerated to generate oxic conditions, and infiltrated or injected for a second time under oxic and carbon-limited conditions [48]. The SMART approach leads to enhanced biotransformation of TOrCs relative to conventional MAR [49] and has been validated at full-scale [30]. The optimal conditions for application of SMART are defined by the oxygen demand of the water, which should be between approximately 8-9 mg/L (saturation in water at 20 °C) and 16−18 mg/L (two times saturation in water). For feed water conditions with constantly higher oxygen demand (*e.g.*, using only partially nitrified wastewater [47]), the second infiltration step cannot be maintained under oxic conditions. Because the biotransformation of some TOrCs is sensitive to minor and short-term changes of redox conditions [30], short-term variations of water quality should be minimized. Other potential practical limitations of SMART include the additional physical footprint for a second infiltration system, cross contamination from vegetation or algae blooms in infiltration basins, and potential clogging and preferential flow paths caused by reduced iron and manganese mobilized in a first anoxic infiltration step (*e.g.*, induced bank filtration, aquifer recharge and recovery). These problems, however, can potentially be managed using new approaches for subsurface trench infiltration [50] and in-situ oxygen injection [51].

Although previous studies clearly demonstrate the potential of SMART to enhance the biotransformation of TOrCs relative to conventional MAR systems, they also

Figure 2



Different strategies to enhance biotransformation of TOrCs.

revealed some general limitations in the biotransformation of TOrCs in MAR systems. For example, some TOrCs exhibit variable extents of biotransformation in different experimental systems despite prevailing oxic and carbon-limited conditions [34]. Other TOrCs, like carbamazepine are persistent in MAR systems (including SMART systems) although its capacity for biotransformation has been shown elsewhere [52]. Biotransformation of apparently persistent TOrCs might be limited by slow reaction rates, specific conditions needed for degrading microbes, the lack of specific degraders or enzymes in microbial communities, or the low chemical concentrations reducing bioavailability for bacteria. New optimization strategies are needed to stimulate the biotransformation of apparently persistent TOrCs.

Other attempts to enhance the biotransformation of different TOrCs may include the concept of habitat heterogeneity. Kassen et al. [53] showed that the productivity and diversity of microbial communities is determined by a heterogeneous environment and [54] suggested to exploit this phenomenon for engineered applications such as bioremediation. In other words, the success of MAR systems compared to activated sludge systems may be related to the heterogeneity of habitats, which is achieved by establishing various redox and substrate gradients. We may also extend such a concept to diverse MAR media that may foster different types of biofilms, facilitating combined TOrC adsorption and biotransformation. The application of highly porous and sorptive filter media has been suggested for biofiltration [55], constructed wetlands [56], and MAR systems [57], but long-term benefits of combined sorption and biotransformation on TOrC removal might be limited [58]. Concepts to manipulate heterogeneity of infiltration zones or the aquifer with the purpose of increasing microbial diversity have not been implemented so far. Such approaches, however, need to be handled carefully as they may also induce clogging or the establishment of preferential flow.

Bioinoculation of MAR systems

One approach to stimulate the biotransformation of apparently persistent TOrCs is to inoculate MAR systems with specialized bacteria, a concept that is well-known from bioaugmentation of contaminated sites

[59]. However, the inoculation of bacteria that biotransform TOrCs is challenging because selective advantages for growth of specific allochthonous bacteria are limited by the limited concentrations of TOrCs and therefore a general lack of ecological selection. Moreover, from a microbial ecology perspective, inoculated strains or more complex coalescing communities (*i.e.*, a naïve engineered community mixed with a resident community) are at a disadvantage unless there are local factors (*e.g.*, new niches in form of carriers or a change in local factors) that rule out priority, propagule, diversity, and overall fitness effects of the established resident community and their interactions [60-63].

While there are no field studies on MAR systems so far, in an analogous system, enhanced biotransformation of 2,6-dichlorobenzamide (BAM) was reported in drinking water sand filters inoculated with specific degrading bacteria [64]. However, removal could not be maintained for longer periods. The period of efficient BAM degradation could be prolonged by providing new niches, when the inoculated degrader strain was immobilized on specific carriers, but low substrate conditions did not support continuous growth in drinking water filters [65]. As more diverse communities are more resistant to less diverse invaders [63], functional degrader communities are theoretically more promising for long-term operations than single strains. Therefore, a niche expansion for functional invaders by *e.g.* material use may facilitate a more long-term success for bioinoculation. One promising new concept maybe to simultaneously expand the metabolic niche by a well designed inoculum. Oña et al. showed that compounds inaccessible to single strains may be used by cross-feeding of the same strains when combined to maintain microbial growth [66]. This metabolic niche expansion was positively correlated with phylogenetic distance, therefore, the design of novel inocula may be most successful with members from diverse phyla and also across domains, a concept that hasn't been tested so far, and that may be particularly relevant for mixed substrate growth described above.

Another concept of bioaugmentation that avoids the problem of invasion failure into an existing community is to provide the functional genes for pollutant degradation to the established microbial community. For example, plasmids may be maintained in a complex microbial community for a longer time without an explicit selection pressure [67]. Moreover, this plasmid persistence may be positively linked to microbial diversity [68]. Similarly, a laboratory pilot study confirmed that communities from rapid sand filter material for groundwater treatment are susceptible for plasmids across many microbial community members [69]. However, early efforts on plasmid bioaugmentation with sequencing batch biofilm wastewater reactors showed that plasmids could only be maintained at lab-scale but not at pilot-scale treatment [70]. This degrader-independent concept is promising and future work could be extended to other mobile elements such as prophages or transposons, however, biosafety regulations often need to be taken into consideration for modified mobile genetic elements and may hamper a broader application under field conditions.

Stimulation of new biotransformation reactions

Different strategies have been discussed to stimulate the biotransformation of TOrCs by either increasing the overall removal potential or by stimulating the activation of specific enzymes involved in biotransformation of TOrCs. To activate promiscuous enzymes for co-metabolic biotransformation, the addition of hardly biodegradable substrates has been suggested [71]. However, more fundamental knowledge on the correlation of refractory organic matter with TOrCs in biological systems, the structural similarities between different substrates and TOrCs, and potential effects of substrate addition are needed to turn this conceptual approach into technical applications. In several studies, removal of TOrCs has been associated with the activity of ammonium monooxygenases (AMO) [35,72], but direct participation of AMO in biotransformation could only be evidenced for few TOrCs [36]. Pre-adaptation strategies with elevated concentrations of a specific TOrC to stimulate the generation of specific enzymes have been proposed, for example, to remove metaldehyde during drinking water biofiltration [73]. The success of this approach depends on the capability of the microbial community to generate TOrC-specific enzymes, and also on the potential to maintain microbial degradation at environmentally relevant concentrations. Besides thresholds for microbial growth, this might also be determined by other factors such as the general bioavailability of the target TOrC or mass transfer limitations. In any case, the spiking of significant amounts of ammonia, individual TOrCs, or other chemicals to enhance biotransformation of TOrCs is not warranted in many cases where drinking water quality may be affected.

Classical ways to increase the microbial biomass and therefore increase the overall removal potential [74], may include the addition of enzymatic cofactors, or the adjustment of the C:N:P ratio to remove the main microbial growth limiters [75]. However, to the best of our knowledge, these concepts still have to be tested in MAR systems.

Conclusions

Specific conclusions of this review include:

- Biotransformation of TOrCs in MAR systems depends on many factors including environmental conditions, substrate availability, and abundance of degrading microorganisms or interactions. Most effective removal for many TOrCs has been reported under oxic conditions.

- Assessment of biotransformation mechanisms, pathways, and relevant enzymes is challenging due to low environmental concentrations of TOrCs and their massive structural diversity. New analytical and experimental concepts are needed.
- Sequential managed aquifer recharge technology (SMART) has been proposed to enhance TOrC biotransformation under controlled oxic and carbon-limited conditions.
- Inoculation of specific TOrC degraders in MAR systems is difficult because low concentrations of TOrCs provide little advantage compared to autochthonous microorganisms. New concepts might involve niche expansion or the introduction of functional genes (on plasmids). These approaches have not been tested in MAR systems.
- Biostimulation by addition of either heterogeneity, growth substrates or co-factors for enzymes is a promising tool in groundwater remediation. Transferability to MAR systems, however, is limited due to the high variability of different TOrCs as well as the potential adverse effects on ground- and drinking water quality from spiking of chemicals such as nitrate or phosphorus.
- The implementation of the aforementioned concepts to enhance biotransformation of TOrCs must always be precluded by consideration of site-specific factors such as subsurface heterogeneity, water flow dynamics, and soil chemistry as these parameters can strongly affect the applicability of individual approaches.
- Besides characterization and optimization of TOrC biotransformation, research is needed to develop concepts to validate reliability of treatment performance in MAR systems. Potential indicator chemicals and surrogate parameters for monitoring of TOrCs biotransformation have been suggested in previous studies [76,77].

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.

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In this study, the authors compared the biotransformation of a large set of TOrCs at different redox and substrate conditions with available information on initial biotransformation reactions.

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