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Bioremediation of pesticide-contaminated water resources: the challenge of low concentrations

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The use of pesticides in agricultural and urban environments has improved quality of life around the world. However, the resulting accumulation of pesticide residues in fresh water resources has negative effects on aquatic ecosystem and human health. Bioremediation has been proposed as an environmentally sound alternative for the remediation of pesticide-contaminated water resources, though full-scale implementation has thus far been limited. One major challenge that has impeded progress is the occurrence of pesticides at low concentrations. Recent research has improved our fundamental understanding of pesticide biodegradation processes occurring at low concentrations under a variety of environmental scenarios and is expected to contribute to the development of applied bioremediation strategies for pesticide-contaminated water resources.

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Introduction

Approximately 2.4 million metric tons of pesticide active ingredients are applied annually worldwide to control the occurrence of weeds, insects, fungi, and other unwanted organisms in agricultural and urban environments [1]. Decades of monitoring studies have documented the occurrence of pesticide residues at trace concentrations (on the order of μg/L and lower) in water resources around the world (e.g. [2•]). One potential pathway of human exposure to pesticides is through drinking water. Even at trace concentrations, pesticides may exceed regulated drinking water concentration thresholds [3] and remediation may be required to protect public health (see Box 1 for summary of key legislation and guidelines for pesticide occurrence in drinking water). Traditional drinking water treatment processes do not effectively remove pesticides from water [3]. Advanced water treatment processes such as activated carbon target pesticides for removal, but are expensive to operate and are not suitable or feasible for all situations [3]. Therefore, alternative strategies are needed to effectively remove pesticides from drinking water resources and limit human exposure.

Engineered bioremediation processes have a long history of application for environmental restoration, however there are unique challenges to consider when designing bioremediation strategies for pesticide-contaminated water resources. Traditional bioremediation processes typically target specific chemical contaminants that are confined in the subsurface at high concentrations. In contrast, bioremediation of pesticide-contaminated water resources must target soluble pesticide residues that are transported in the aqueous phase at low concentrations. Further, pesticides occur in water resources along with other carbon substrates that are present at similar or greater concentrations; pesticide degraders must compete with indigenous microbial communities for these carbon substrates while maintaining biodegradation activity towards pesticides. In this review, the recent literature on pesticide biodegradation and bioremediation is explored while focusing on these unique challenges. First, the kinetic and physiological factors that determine the extent of pesticide biodegradation under a variety of environmental scenarios are considered. Then, the general strategies that have been proposed for the bioremediation of pesticide-contaminated water resources are introduced. Finally, the main challenges limiting the application of specific techniques are discussed.

Factors that determine the extent of pesticide biodegradation

Biodegradation is regarded as the most important means for natural attenuation of pesticides in the environment [9]. However, pesticide biodegradation will only occur under favorable environmental conditions [10]. One critical factor that determines the extent of pesticide biodegradation is the interaction between the pesticide degrader and the indigenous microbial community along with the consequent competition for other assimilable organic carbon (AOC) substrates. These interactions are presented schematically in Figure 1. There are two limiting scenarios that can reduce the complexity of these interactions. The first scenario is delineated on the left side of Figure 1 and is characterized by relatively high growth

Box 1 Summary of key legislation and guidelines for pesticide occurrence in drinking water

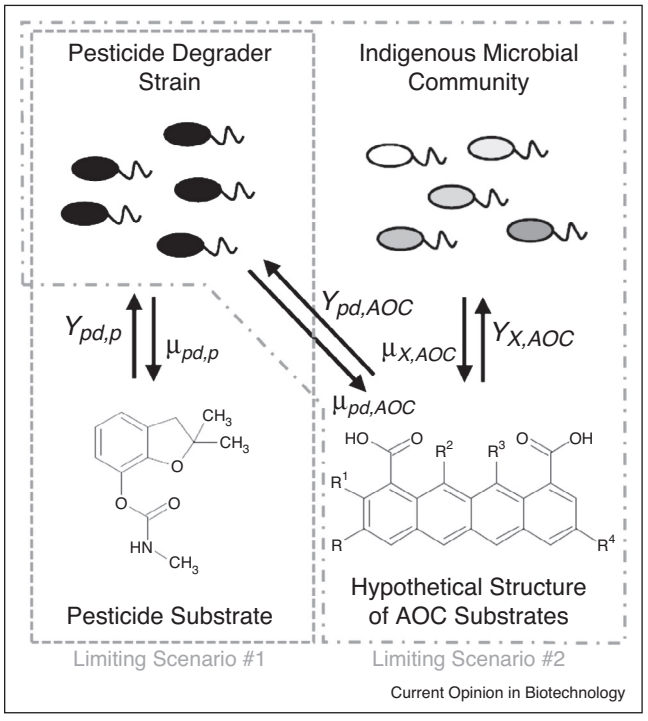
World Health Organization (WHO) Guidelines for Drinking Water Quality [4]
- Establishes guideline values (GVs) for 32 individual pesticides that are of health significance in drinking water.
- GVs range between 0.03 and 200 μg/L.

European Union (EU) Groundwater Directive [5]
- Stipulates maximum allowable concentration of all individual pesticides in drinking water is 0.1 μg/L.
- Stipulates that the sum of all pesticide concentrations is less than 0.5 μg/L.
- Stipulates maximum allowable concentration of aldrin, dieldrin, heptachlor, and heptachlor epoxide is 0.03 μg/L.

Australian Drinking Water Guidelines 6 [6]
- Establishes guideline values (GVs) for 153 individual pesticides.
- GVs range between 0.0003 and 9 μg/L.

United States Environmental Protection Agency (USEPA) Safe Drinking Water Act
- Stipulates maximum contaminant levels (MCLs) for 21 individual pesticides [7].
- Stipulates MCLs for the 21 pesticides in the range of 0.2–700 μg/L.
- Identifies 43 additional pesticides and pesticide degradation products on a contaminant candidate list (CCL) that may require an MCL in future regulations [8].

Figure 1



Schematic of interactions between pesticide degraders and indigenous microbial communities along with the consequent competition for other assimilable organic carbon (AOC) substrates. Key parameters that determine the extent of pesticide biodegradation are the growth rate and yield of the pesticide degrader on the pesticide ($\mu_{pd,p}$, $Y_{pd,p}$), the growth rate and yield of the pesticide degrader on other AOC substrates ($\mu_{pd,AOC}$, $Y_{pd,AOC}$), and the growth rate and yield of the indigenous microbial community on other AOC substrates ($\mu_{X,AOC}$, $Y_{X,AOC}$). The limiting scenarios described in the text are delineated by the dashed lines. Schematic is adapted from Liu et al. [18•].

rates of the pesticide degrader on the pesticide substrate. This can occur when pesticide concentrations are high or when enzyme affinities for the target pesticide are greater than enzyme affinities for other AOC substrates. For example, agricultural soils are generally characterized by high concentrations of pesticides following application; under these conditions, significant biodegradation and mineralization of pesticides has been reported (e.g. [11]). Bacterial enzymes may also evolve in response to prolonged exposure to high concentrations of specific pesticides which can lead to the construction of novel metabolic pathways [12] or enhanced metabolic activity [13]. In this limiting scenario, interactions with the indigenous microbial community or other AOC substrates are not expected to have a significant effect on pesticide biodegradation. The second scenario is delineated on the right side of Figure 1 and is characterized by relatively high growth rates of either the pesticide degrader or the indigenous microbial community on other AOC substrates. This can occur when the concentration of AOC substrates is high or enzyme affinities for the target pesticide are relatively low. For example, wastewater treatment plant influents are generally characterized by high concentrations of other AOC substrates and low concentrations of pesticides [14]. Under these limiting conditions, the environment selects for microorganisms that grow on the abundant AOC substrates and pesticides are typically recalcitrant [14]. Pesticide removal reported in these types of environments is generally attributed to fortuitous metabolism [9] evidenced by the formation of pesticide degradation products [15].

Pesticide-contaminated water resources are not generally characterized by one of these limiting scenarios and therefore the complement of interactions presented in Figure 1 are important for determining the extent of pesticide biodegradation. The co-occurrence of indigenous microbial communities and other AOC substrates can have either positive or negative effects on the extent of pesticide biodegradation [16,17]. A recent model developed and validated using literature reported biodegradation data demonstrated that the effects of interactions with indigenous microbial communities or other AOC substrates can largely be predicted by considering the kinetics of those interactions [18•]. The key parameters

needed to make predictions on the extent of pesticide biodegradation are the growth rates and yields of the pesticide degraders and the indigenous microbial community on the pesticide and other AOC substrates [18•]. These parameters are difficult to measure in biodegradation experiments conducted at environmentally relevant concentrations. However, two methods based on high accuracy cell counting and experimental procedures that minimize interferences from external carbon were recently developed [19,20••]. Application of these methods has led to robust estimates of growth rates and yields of pesticide degraders in experiments conducted at low concentrations [20••]. Importantly, growth rates measured at relatively high pesticide concentrations could predict growth rates measured at low concentrations [20••]. These new methods can be applied to estimate kinetic parameters for growth on a variety of pesticide and AOC substrates. Fully parameterized models can be used to simulate pesticide biodegradation under a wide range of environmental scenarios and to optimize bioremediation processes [18•].

A variety of physiological processes are also important for pesticide biodegradation at low concentrations. For example, some pesticide degraders can mineralize target pesticides at environmentally relevant concentrations by means of a constitutively expressed catabolic pathway [21]. In contrast, other pesticide degraders have one or more catabolic genes in the pathway that require induction at higher concentrations [22] which can lead to recalcitrance or the accumulation of biodegradation intermediates. Catabolic gene induction may also play a role in the phenomenon of threshold concentrations which are often reported in the range of 1–100 μg/L [17], though the specific causes of threshold concentrations remain poorly understood. Carbon catabolite repression may also be an important physiological process affecting pesticide biodegradation in environments with varying types and amounts of other AOC substrates. In low concentration environments, mixed substrate utilization has been widely reported (e.g. [17]). However, recent data showed that the specific activity of a pesticide degrader was suppressed in the presence of easily degradable AOC substrates at low concentrations [23•]. Other experiments likewise showed that the extent of biodegradation of a variety of trace organic contaminants (including pesticides) was suppressed as the quantity of easily degradable AOC substrate supplements was increased [24,25]. These data suggest that carbon catabolite repression may be relevant under certain environmental scenarios and an important consideration in developing bioremediation strategies.

Bioremediation of pesticide-contaminated water resources

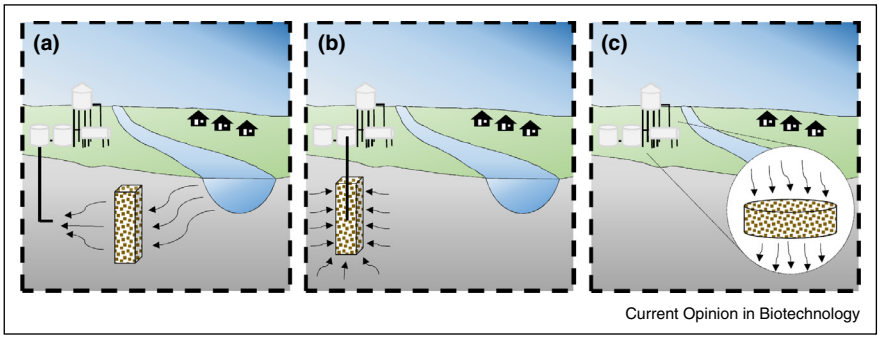
Most bioremediation strategies considered for pesticide-contaminated water resources involve biofiltration. There are two main reasons for this. First, pesticides in water resources are generally mobile. Biofiltration enables the design of a confined bioremediation compartment through which pesticide-contaminated water can flow. Second, the hydrodynamics of some types of biofiltration systems can be controlled in such a way that enables high loading rates of pesticides and other carbon substrates even when they are present at low concentrations. High loading rates can have positive effects on biodegradation by generating high fluxes of substrates that consequently enhance metabolic activity, though high loading rates can also limit hydraulic residence times which could have negative effects on bioremediation. Some general bioremediation schemes for pesticide-contaminated water resources related to drinking water production are provided in Figure 2. Natural attenuation of pesticides in experimental biofiltration systems has been reported (e.g. [26]), but biodegradation is most often incomplete resulting in either residual concentrations of pesticides or pesticide degradation products in effluents. Therefore, the aim of bioremediation is to optimize the rate and extent of biodegradation by employing techniques such as bioaugmentation and biostimulation.

Bioaugmentation in pesticide-contaminated water resources

Bioaugmentation involves application of non-native degraders to a contaminated environment or engineered process to enhance the biodegradation of target chemical contaminants. Bioaugmentation has been explored at laboratory-scale and pilot-scale to remove taste and odor compounds [27] and triazine pesticides [28,29] during drinking water production. More recently, bioaugmentation has been successfully demonstrated for the remediation of soils containing a variety of pesticides including endosulfan, 4-chloro-2-methylphenoxyacetic acid (MCPA), and linuron [30–32]. There are two main challenges in developing bioaugmentation strategies for the targeted removal of pesticides in water resources. First, suitable pesticide degraders must be isolated with the requisite metabolic capabilities and physiology to utilize the target pesticides at low concentrations and under a range of environmental scenarios. Second, the pesticide degrader must remain in the bioremediation compartment and maintain activity towards the target pesticide over extended time scales.

Pesticide degraders are generally isolated from environmental samples following selective enrichment on relatively high concentrations of the target pesticide [33–35]. However, it was recently demonstrated that the physiology of the resulting degraders can be dependent on the conditions at which they were enriched [36••]. Specifically, microbial communities that could utilize MCPA were enriched from groundwater on low and high concentrations of MCPA [36••]. The results showed that both communities had biodegradation activity towards MCPA, but the community isolated at low MCPA concentrations had greater activity towards low concentrations of MCPA as evidenced by shorter lag phases [36••]. Thus, a subpopulation of

Figure 2



General bioremediation schemes for pesticide-contaminated water resources at various locations within a water system. **(a)** Biofiltration is used as part of managed bank filtration. A bioremediation zone is designed and optimized for remediation of pesticide-contaminated water prior to reaching the intake at the drinking water treatment plant. **(b)** Biofiltration is applied directly at the intake to the drinking water treatment plant. Here, pesticide-contaminated groundwater is treated prior to the traditional treatment train. **(c)** Biofiltration is applied in conjunction with traditional filtration objectives within the drinking water treatment plant. In each example, bioaugmentation or biostimulation could be used to enhance biodegradation of pesticides.

MCPA degraders was isolated that might be better suited for bioaugmentation in water resources contaminated with low concentrations of MCPA. Fungi are also recognized as important pesticide degraders. Fungal degraders have recently been isolated that can biodegrade organophosphate pesticides [37,38] and pesticides containing aromatic amine functional groups [39]. Mixed bacterial and fungal communities have also shown cooperative improvements to pesticide biodegradation. For example, diuron and 2,6-dichlorobenzamide (BAM) were mineralized more rapidly in sand when bacterial and fungal degraders were simultaneously present than when the bacterial degraders were present alone [40,41]. These observations were attributed to cooperative metabolism or enhanced transport of bacterial degraders by fungal hyphae [41].

In lieu of selective enrichment for identifying novel pesticide degraders, others have considered investigating the metabolic capabilities of organisms that are known to metabolize anthropogenic chemicals that have analogous chemical structures to pesticides. For example, a pair of bacteria that use lactonases to oxidize a variety of oil constituents were recently shown to metabolize organophosphate pesticides using the same enzymes [42]. Further, laboratory techniques were used to enhance the catalytic activity of lacotonases towards organophosphate pesticides through induced mutations [43•]. The isolated mutants lost their activity towards lactones, but showed increased affinity towards a number of organophosphate pesticides [43•]. In a separate example, a bacterium that metabolizes polycyclic aromatic hydrocarbons was used to metabolize the carbaryl group of N-methylcarbamate pesticides [44]. Another emerging approach is to expand the metabolic capacity of pesticide degraders. For example, a genetically engineered microorganism capable of simultaneously degrading organophosphate and organochlorine pesticides was constructed by display of organophosphate hydrolase on the cell surface of a hexachlorocyclohexane (HCH)-degrading bacterium [45]. The modified organism showed simultaneous activity towards organophosphate and organochlorine pesticides. In sum, these techniques are expected to contribute to identifying pesticide degraders for bioaugmentation, though it remains critical to investigate degrader physiology at low concentrations and in the presence of indigenous microbial communities and other AOC substrates when targeting water resources contaminated with low concentrations of pesticides.

Approaches to ensure successful invasion of a pesticide degrader into an indigenous microbial community are limited. Recent work aimed at understanding the potential for pathogen proliferation in drinking water systems revealed that pathogens were outcompeted by native microbial communities adapted to life in drinking water [46,47]. These results reinforce the recommendation to isolate pesticide degraders from environments and under conditions that are similar to those in which the bioaugmentation will occur [36••]. Other studies have looked at ecological factors that control bacterial invasion of microbial communities. For example, it was demonstrated that microbial communities with a high level of evenness are more resistant to invasion due to greater niche overlap among the native taxa [48]. Therefore, an understanding of the community composition into which bioaugmentation is planned is also expected to be an essential prerequisite for understanding potential success. If a successful invasion is unlikely or unexpected, an alternative approach could be to encapsulate or immobilize pesticide degraders onto surfaces. This can protect cells from predators, prevent washout, and consequently extend the lifetime of biodegradation activity. For example, the biodegradation capacity of a lindane degrader persisted longer in liquid and slurry microcosms when encapsulated

in open-ended tubes which resulted in a slow release of active lindane degraders [49]. The duration of biodegradation activity was also extended following encapsulation of degraders in polyvinyl alcohol cryogel beads [50], calcium alginate beads [51], and other natural materials [52]. These immobilization techniques are expected to significantly enhance the vitality and lifespan of a bioaugmentation system.

Biostimulation in pesticide-contaminated water resources

Biostimulation is a bioremediation technique that involves enhancement of microbial community activity following manipulation of the physicochemical environment. This is most often accomplished by adding various forms of potentially rate limiting substrates or nutrients to the environment. For example, bentazone, mecoprop, and dichlorprop biodegradation was stimulated in anaerobic aquifer material following the addition of oxygen [53]. A new approach to biostimulation could be to manipulate a microbial community to enhance biodegradation activity towards a broader range of pesticides. There are at least two ways that this could be achieved. First, certain measures of biodiversity including taxonomic richness have been shown to associate positively and significantly with the collective biotransformation rates of multiple trace organic pollutants in microbial communities [54]. Taxonomic richness was enhanced in laboratory-scale experiments by providing niche opportunities for microorganisms in the form of physical and chemical heterogeneities [55]. Manipulating the taxonomic richness of microbial communities in engineered bioremediation processes by providing niche opportunities may result in greater biodegradation activity towards a broader range of pesticides. Second, microorganisms generally express larger numbers of catabolic genes under oligotrophic conditions [56]. This physiological mechanism allows bacteria to take advantage of a broad range of scarce substrates in oligotrophic environments. Constructing biofiltration systems that treat contaminated water in a series of compartments could lead to increasingly oligotrophic bioremediation zones and microbial communities with greater metabolic activity, though it remains unclear whether catabolic enzymes with specific pesticide activity would be stimulated in this way. These strategies that exploit our fundamental understanding of microbial community ecology could prove useful in developing bioremediation strategies that simultaneously remove complex mixtures of pesticides in water resources.

Conclusion

Bioremediation is a promising technology for remediation of pesticide-contaminated water resources. Traditional techniques such as bioaugmentation and biostimulation are expected to contribute to successful solutions, but application of these techniques must be preceded by careful consideration of the unique challenges posed by pesticide contamination. Environments in which bioremediation is planned should be fully characterized in terms of the activity of the indigenous microbial community and the occurrence of other AOC substrates to enable performance predictions for a variety of proposed bioremediation options. Continued research should focus on improving our fundamental understanding of kinetic, physiological, and ecological processes occurring in low concentration environments. Advances in these areas are expected to lead to new approaches to effectively design and optimize bioremediation strategies for pesticide-contaminated water resources.

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