

Feasibility of Using Bacterial-Microalgal Consortium for the Bioremediation of Organic Pesticides: Application Constraints and Future Prospects



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1 Introduction

A pesticide is a chemical compound designed to kill pests including weeds, fungus, insects, and rodents, as defined by the World Health Organization, but has been expanded by the Food and Agriculture Organization of the United Nations to include any chemical designed to control disease vectors for human and animals, as well as any pest threatening agricultural or industrial commodities (Li and Jennings 2017). Pesticides can be defined by both the active ingredient designed to control pests and any additional ingredients designed to improve the efficacy of the active ingredient such as emulsifiers or fumigants. Pesticides are often categorized by the targeted organism and range from avicides, rodenticides, insecticides, miticides (acaricides), molluscicides, nematicides, herbicides, fungicides, algicides, bactericides, and viricides with the prefix of each category describing the target (Uqab et al. 2016). However, this chapter focuses on four main delineations of organic pesticides, based on the chemical structure and associated mechanism of action of the active ingredient: organochlorines, organophosphates, carbamates, and pyrethrin or pyrethroids (Li and Jennings 2017). Each of these pesticide classes has been expanded to include many different isomers and related compounds, but each class has a specific mechanism affecting pests that also make them toxic to other species.

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2 Pesticide Production and Use

Pesticide use is justified through overall increases in crop yield and reductions in postharvest losses, thus improving food security and lowering overall costs (Damalas and Eleftherohorinos 2011). However, the persistence of these compounds in the ecosystem is associated with a litany of negative effects on environmental security, water security, and human health (Aktar et al. 2009; Fukuto 1990; Uqab et al. 2016). Furthermore, the unintentional by-products of the production of some of these compounds also pose ecological and human health risks, as they have historically found their way into the environment (FAO 2018).

Over time, the production and use of some pesticide classes and their associated compounds became heavily regulated in the developed world, yet many developing countries don't have the means to control or regulate their use (Alavanja 2009). Large stockpiles of expired compounds referred to as "obsolete pesticides" are causing widespread contamination of soils and surface waters in the developing world (FAO 2018). However, when one group of pesticides is outlawed, a new group soon replaces them. For instance, since the introduction of "round-up ready" genetically modified crops in the 1990s, the use of glyphosate (organophosphate herbicide) has risen dramatically and won't likely decrease any time soon; it is believed that the accumulation of this moderately persistent chemical and the associated metabolites will continue to accrue in aquatic systems and soils (Benbrook 2016; Kniss 2017).

2.1 Pesticide Types and Mechanisms

Organochlorine Pesticides

Organochlorines are chlorinated carbon compounds that were once used worldwide and are considered the first generation of pesticide chemicals. This class of compounds includes hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), and the endocrine disruptor endosulfan. Organochlorines are extremely resistant to environmental degradation and have been labeled as persistent organic pollutants (POPs) by the United Nations Stockholm Convention. Even though these chemicals are heavily regulated throughout the developed world, they are still used throughout parts of Asia and various members of the developing world as a public health measure against malaria spread (Jayaraj et al. 2016). Organochlorines such as DDT prevent the closing of sodium ion gates at the axon terminal of neurons resulting in a negative membrane potential causing repeated neural discharges (Coats 1990). Chlorinated cyclodienes like aldrin, chlordane, and endosulfan are neurotoxic based on the chemical binding affinity of the picrotoxin site of the γ -aminobutyric acid chloride ionophore complex (GABA), thereby disrupting neural intake of chloride anions (Coats 1990). These chemicals can wreak havoc to the endocrine system in mammals and are associated with a wide variety of health

defects (Jayaraj et al. 2016). Organochlorine pesticide usage has been heavily regulated and even banned due to this off-target toxicity, prominent levels of bioaccumulation, and notorious persistence in the environment (Coats 1990; Jayaraj et al. 2016; Katagi 2010).

Organophosphates and Carbamates

The next generation of pesticides were derived from esters of phosphoric acid and are called organophosphates. The toxicity of organophosphates is identical to that of carbamate esters, derivatives of carbamic acid. Organophosphates and carbamate esters inhibit the hydrolase activity of acetylcholine hydrolase (AChE) on the neurotransmitter acetylcholine (Fukuto 1990). AChE activity is necessary in both vertebrate and invertebrate organ systems and is nearly ubiquitous in parasympathetic nervous systems and is responsible for the rapid hydrolysis of acetylcholine into acetic acid and choline (Fukuto 1990). Organophosphates include glyphosate, the most commonly used pesticide in the United States since 2001, with 1.22–1.32 × 10⁵ tons applied in 2012 alone (Atwood and Paisley-Jones 2017).

Pyrethroids and Other Pesticides

Pyrethroids are synthetic derivatives of naturally occurring insecticidal compounds produced by *Chrysanthemum* spp. and have twofold mechanisms of action: (i) inhibiting sodium ion channels in neuron membranes and (ii) inhibiting the GABA complex (Coats 1990). The lipophilicity of pyrethroids increases bioaccumulation along food chains and is associated with long-term exposure problems even though they are less likely to persist in the environment abiotically (Tang et al. 2018).

Neonicotinoids were developed as a replacement for organophosphates and represent a systemic approach to controlling insects; the pesticide is taken up by the plant through root diffusion where it then spreads to all parts of the plant (Cimino et al. 2017).

2.2 Spatial and Temporal Quantification of Production and Use

The world population is predicted to exceed 9 billion by 2050; the use of pesticides is necessary and justified to ensure food security for the impending population increase (Bonner and Alavanja 2017). It is estimated that nearly 40% of agricultural production is protected through the use of pesticides (Senthil Kumar et al. 2018). Furthermore, insecticides have become an important tool in controlling insect vectors of disease including mosquito species associated with Zika, West Nile virus, dengue, yellow fever, and malaria (Lawler 2017).

Quantifying pesticide production has proven challenging due to self-reporting issues and unauthorized use, but all figures indicate drastic increases since initial use in the middle of last century. The first pesticide use survey in the United States was conducted in 1964, and within 20 years, usage grew from 48 million pounds (21.8×10^3 tons/yr) to 430 million pounds (19.5×10^4 tons/yr) of active ingredient alone (Osteen and Fernandez-Cornejo 2016). Pesticide use is estimated to have steadily risen 11% annually, worldwide since the 1950s helping to support the exponential population growth through both disease vector control and overall crop production (Carvalho 2017). By the year 2000, an estimated 5 million tons of pesticides were being produced every year (Carvalho 2017). However, in the years that followed, the US Environmental Protection Agency's consumer-based studies show more conserved trends. An estimated 6 billion pounds (2.7×10^6 tons/yr) of pesticides were used annually, worldwide in 2011 and 2012 with the United States being responsible for 1.1 billion pounds (5.0×10^5 tons/yr) (Atwood and Paisley-Jones 2017). The most recent estimates provide that pesticide production has risen again to 3.3×10^6 tons/yr with Europe being responsible for 4.2×10^5 tons/yr (Hvězďová et al. 2018).

In the United States, herbicides such as glyphosate, atrazine, and S-metolachlor make up the bulk of current agricultural industrial use (57%), while fumigants (37%), fungicides (9%), and insecticides (5%) account for nearly the rest (Atwood and Paisley-Jones 2017). This ratio of herbicides and fungicides making up the bulk of pesticide use is mirrored throughout much of the developed world. However, 76% of India's pesticide use is attributed to insecticides, while nearly two thirds of all pesticides used were DDTs and HCHs (Yadav et al. 2015). It stands to reason that areas with more tropical climate consume higher levels of insecticides, likely for crop protection and public health measures against disease associated insect vectors.

Current paradigm of use and high rates of persistence are associated with ecological contamination often leading to human health crises. Conventional attempts at remediation are costly, environmentally hazardous, and often ineffective. Biological remediation has been investigated for decades as an efficient methodology for remediating contamination of water and soil. These methods have traditionally focused on the bacterial remediation of organic pollutants; however, microalgal-bacterial consortiums have shown great potential for the biological remediation of pesticides (Uqab et al. 2016). This chapter discusses the fate of pesticides in the environment and the associated health risks, former applications of microalgae and bacteria, inter-kingdom synergies, and factors affecting and limiting the efficacy of bacterial-algal bioremediation of pesticides.

3 Environmental Fate and Ecological Risk of Pesticides

Some studies have provided that only 0.1% of all pesticides reach their target organism, meaning the resulting 99.9% are left to enter the environment (Pimentel 1995). Even following correct application, many of these compounds enter the

ecosystem through water runoff events leading to the contamination of surface water, groundwater, estuaries, marine environments, and soil deposits often persisting for prolonged periods of time. A recent study of arable soils in Europe, months after application, detected pesticides above the risk levels for the regions containing suspected carcinogens and endocrine disruptors (Hvězdová et al. 2018). These chemicals are then either broken down by photolytically, biologically, or chemically; if they aren't degraded, they persist in the water cycle or are adsorbed by other organisms, thus entering the food chain (Senthil Kumar et al. 2018). Some chemicals are highly persistent due to the chemical compound's structural resistance to abiotic or environmental degradation. For example, organochloride pesticides such as DDT and associated derivatives have half-lives ranging from 2 to 15 years (Jayaraj et al. 2016). Highly persistent chemicals undergo evaporation and condensation in the water cycle traveling immense distances (Subashchandrabose et al. 2013). This long-range atmospheric transport of persistent organic pesticides shows that pesticide pollution is not relegated to certain countries or regions; the pollutants and their associated harm are shared through geochemical processes (Yadav et al. 2015).

While some pesticides are not present in water or soils in large enough concentrations to do harm, the lipophilicity of some pesticides can lead to their accumulation in living organisms and subsequent vertical transfer through the food chain and are known as bioaccumulation and biomagnification (Katagi 2010). Even more readily degraded pesticides such as glyphosate have been shown to be accumulating in large amounts over many years, resulting in risking contamination of water supply and food stuffs beyond human use (Carvalho 2017).

4 Human Health Risks

Human exposure to pesticides should be avoided because they are, by nature, hazardous as they are designed and manufactured to be toxic. Humans generally come into contact with pesticides in three ways: (i) during the production or use of pesticides, (ii) through ingestion of food or water contaminated with pesticides, and (iii) through inhalation of pesticide-contaminated air or through skin contact with contaminated water or soil. Acute exposure toxicity is well understood and more defined than low-dose long-term exposure because of the complexity of studying long-term toxicological mechanisms (Bonner and Alavanja 2017).

4.1 Acute Pesticide Toxicity

Persons at highest risk for exposure to pesticides are those who directly handle pesticides and include pesticide applicators, production workers, and farm workers (Alavanja 2009). Studies have shown that members of the developing world are disproportionately at higher risk of acute pesticide poisonings, especially in rural

areas (Eddleston 2016). Contributing factors include improper handling and storage regulation, reduced access to personal protective equipment (PPE), and reduced access to adequate health care (Alavanja 2009; Yadav et al. 2015). For instance, one study compared the levels of a chlorpyrifos metabolite in urine samples between pesticide applicators without PPE to adolescents who were non-applicators finding a nearly tenfold increase (Bonner and Alavanja 2017). Organophosphate insecticides are estimated to cause nearly two million hospitalizations and resulting in nearly 100,000 deaths yearly in the developing world (Eddleston 2016). One review detailed that acute poisonings were 13-fold higher in the developing world compared to industrialized countries (Aktar et al. 2009). Another review detailed numerous studies showing the relationship between neurological disorders and exposure to HCH, as well as cardiotoxic symptoms related to methomyl application (Aktar et al. 2009), with dermal absorption likely being the method of exposure (Kim et al. 2017). While pesticide poisonings are a concern, they are predicted to decrease as safer pesticides or non-pesticidal control chemicals such as methoprene enter the market. However, long-term exposure to pesticide residues through indirect exposure is of great concern worldwide. Various regulatory bodies have prevented the developing and marketing of genotoxic pesticides through in vitro model systems, and widespread epidemiological and cohort studies have proven increased risk of cancer, diabetes, birth and development disorders, asthma, and neurodegenerative disorders (Jayaraj et al. 2016; Kamel and Hoppin 2004; Kim et al. 2017). More studies are required to assess the exposure of currently used pesticides over extended periods of time comparatively in order to work out the mechanisms of non-genotoxic carcinogenic pesticide residues, i.e., those affecting chromatin remodeling and other epigenetic effects (Alavanja 2009).

4.2 Long-Term Exposure Toxicity

Exposure to widely used pesticides has been associated with cancer, endocrine disruption, and neurological disorders (Carvalho 2017; Kamel and Hoppin 2004). Pesticides such as γ -HCH and DDT are associated with immunosuppressive effects, causing oxidative stress in blood cells, and even stimulate cancer cell propagation through in vitro studies (Bonner and Alavanja 2017). Endosulfan is associated with immunosuppression, disruption of spermatogenesis, and sperm morphology and also causes damage and mutation to DNA (Jayaraj et al. 2016). Long-term pesticide exposure meta-analysis revealed a relationship between exposure and occurrence of hematological malignancies such as Hodgkin's lymphoma (Hu et al. 2015).

The prevalence of pesticide use, their environmental fate, and associated human health effects indicate a need to develop novel approaches to remediating the environment to protect humans and ecosystems from pesticide degradation.

5 Common Biological Approaches to Remediation

Pesticide usage and the persistence of the compounds have negative implications in the realms of ecology and human health. However, conventional cleanup methods attempting to solubilize and recover organic compounds are costly and ineffective. Many of these cleanup attempts require significant investment to infrastructure and are not self-maintained, leading to a high cost with a low cost-benefit ratio (Velázquez-Fernández et al. 2012; Xia et al. 2012). Furthermore, these conventional remediation techniques are not ecologically friendly and even increase the risk of further environmental contamination. There has been a recent push toward biologically based remediation practices for the efficiency and cost-effectiveness.

The concept of bioremediation involves the exploitation of already existing mechanisms employed by bacteria, fungi, algae, and higher plants to detoxify, degrade, or accumulate pollutants to be later removed. The bioremediation of pesticides can occur *in situ* where the pesticides are degraded or accumulated at the location of pollution or *ex situ* where the contaminated soil or water is extracted and relocated to a different site for treatment (Senthil Kumar et al. 2018). Both processes *conventionally* use microbes isolated from the location of the pollution to achieve the desired biodegradation or transformation. The concept of bioaugmentation is an *in situ* approach where already existing flora are augmented to improve or facilitate the desired remediation. Whether the remediation occurs *in situ*, on site, or *ex situ*, the combination of bacteria and microalgae has great potential for the remediation of organic pesticides. The following section discusses the potentials and application examples.

6 Application of Bacterial-Microalgal Consortium

The efficacy of bacterial-microalgal consortia for the bioremediation of pesticides is based in the ecological associations between bacteria and algae but likely has roots in an evolutionary context as well (Ramanan et al. 2016). Microalgae and bacteria are the largest communities of primary producers across every type of aquatic ecosystem and play a major role in the aquatic carbon cycle but also play a role in terrestrial carbon cycling (Ramanan et al. 2016). Over 200 million years of coevolution have provided a number of inter-kingdom synergistic relationships including inter-kingdom quorum signaling, interspecies biofilm formation, and especially co-metabolism (Amin et al. 2012).

Microalgae have proven effective at accumulating pesticides once they've entered aquatic and terrestrial ecosystems. While some species are capable of complete mineralization or transformation of pesticides into less toxic metabolites, some species are inhibited by the toxic effect of said metabolites. Therefore, it stands to reason that by pairing these accumulators with bacterial degraders, the

overall efficacy of bioaugmentation is radically improved. Here, we discuss the potentials and applications of a bacterial-microbial consortium for the bioremediation of pesticide pollution.

6.1 *Microalgae Accumulation and Transformation*

One study was able to show that ten distinct species of microalgae and cyanobacteria were capable of oxidizing the organophosphate nematicide fenamiphos, while *Chlorella* sp. and *Anabaena* sp. were able to detoxify 99% of the chemical (Cáceres et al. 2008b). The same group used freshwater *P. subcapitata*, a freshwater algae and soil algae *Chlorococcum* sp. to accumulate and transform fenamiphos by 100% and 62%, suggesting that liquid suspension might be more effective (Cáceres et al. 2008a). *Anabaena azotica* isolated from rice paddies showed tolerance and bioremediation degradation potential of γ -HCH (Lindane) by removing nearly 50% in 5 days (Zhang et al. 2012). One study observed a random mutation that allowed a species of *S. intermedius* to develop resistance to lindane and even showed potential for its removal from aquatic systems (González et al. 2012). Both *Chlorococcum* sp. and *Scenedesmus* sp. were able to degrade endosulfan in both liquid media and soil (Sethunathan et al. 2004).

6.2 *Bacterial Degradation*

The basis for all degradation and transformation of pesticide pollutants begins with the necessary enzymatic activity and is separated into three classes. Enzymes modifying functional groups, enzymes associated with transfer reactions of whole groups to pollutants, and enzymes capable of translocation making pollutants unavailable to organisms are classified as phase I, II, and III enzymes, respectively (Velázquez-Fernández et al. 2012). These are typically transferase, oxidoreductase, and hydrolase enzymes. The use of consortiums to degrade complex organic molecules has been proven effective in many bioaugmentation studies (Mrozik and Piotrowska-Seget 2010). The combination of catabolic pathways of different organisms greatly enhances the overall efficacy of bioremediation. In addition, by using a consortium of bacteria from an already contaminated site, the overall efficiency of degradation is improved because there is less overall accumulation of toxic compounds and metabolic waste (Pino and Peñuela 2011). For instance, chlorpyrifos and methyl parathion were both effectively degraded by a bacterial consortium obtained from contaminated soils in Columbia (Pino and Peñuela 2011). Another study used autochthonous microbial consortiums capable of degrading organophosphates in soil, which were then inoculated with *Serratia marcescens*, thereby reducing remediation times by 8–20 days dependent upon soil types (Cycoń et al. 2013).

The combination of the transformation potentials of various microalgae with the degradation potentials of heterotrophic bacteria and the overall efficiency of bioremediation can be greatly improved. As long as there are no unintended interactions

involving predation, resource competition, or metabolite toxicity, the algae-bacteria consortiums can exhibit effective remediation of organic pesticides. This has been proven effective in a few cases. The following section highlights some effective consortiums

6.3 Examples of Effective Consortiums

Microalgae-bacterial consortiums have been used for half a century for the removal of nutrients from wastewater, agro-industrial effluent, and heavy metal contamination (Ramanan et al. 2016). Surface water, groundwater, effluent, and even soils contaminated with pesticides can all be remediated with microalgal-bacterial consortiums. Many pilot scale studies have quantified the rate at which pesticides are accumulated or transformed by algal-bacterial consortiums. These organisms are often isolated from an area with high levels of pesticide contamination, are then cultured, and are used in combination with other microbes for the biodegradation or bioaugmentation of organic pollutants (Cycón et al. 2017; Velázquez-Fernández et al. 2012; Yañez-Ocampo et al. 2009; Zhao et al. 2015). One review compiled over a decade of studies where a consortium of *Chlorella* sp., *Selenastrum* sp., *Phormidium* sp., and *Scenedesmus* sp. of microalgae were used in tandem with varieties of proteobacteria (*Ralstonia*, *Pseudomonas*, *Burkholderia*, *Sphingomonas*, *Acinetobacter*) and actinobacteria (*Rhodococcus*, *Mycobacterium*) to effectively degrade a wide variety of organic pollutants (Subashchandrabose et al. 2011). Microalgae produce O₂ which is used by the aerobic bacterial strains for the mineralization of the organic compounds (Muñoz et al. 2006). This type of co-metabolism can feasibly be exploited during the biodegradation of organic pesticides as well, with the main limitation being toxicity to microalgae strains. There are many factors that affect the rate at which a microorganism can degrade or assimilate a pesticide. These factors include toxicity of the pesticide, pH, sunlight, temperature, and endogenous metabolism (Subashchandrabose et al. 2011, 2013). These factors can be easily controlled in a lab setting but need to be taken into consideration when designing in situ remediation.

6.4 Genetic Modifications in Bioremediation

A molecular understanding of the consortium metabolism can be used to genetically manipulate members of the microbial consortium to improve the remediation and augmentation. The enzyme family cytochrome P450 monooxygenase (CYPs) have been used for the degradation of polyaromatic hydrocarbons with wild-type enzymes from *Bacillus* and *Pseudomonas* species being modified to improve degradation activities (Gaur et al. 2018). The use of genetically altered bacteria and/or algae can provide fitness increases or differential stress responses through upregulating or modifying enzymatic activity, alleviating the rate-limiting steps in a metabolic

pathway, or even enhancing energy production inside communities (Ortiz-Hernandez et al. 2013).

An algal strain of *Sphingobium japonicum* capable of HCH degradation was genetically engineered to display an organophosphate hydrolase enzyme from the bacterium *Pseudomonas syringae* (Cao et al. 2013). This study observed that the engineered organism could degrade parathion amounts of 100 and 10 mg/kg of lindane (γ -HCH) completely within 15 days (Cao et al. 2013). This example of genetic engineering completely circumvents microalgae-bacterial consortium use through combining one capable organism with the enzyme capabilities of bacteria. While these methods show immense potential for designing an organism to fit individual pollution sites, it is costly and time-consuming. Furthermore, the induced mutations may be energetically unfavorable to the organism and may lead to fitness decreases outside the laboratory (Gaur et al. 2018). Therefore, it is often easier and more cost-effective to use a symbiotic consortium of microalgae and bacteria.

6.5 Factors Affecting Pesticide Removal by Bacterial-Microbial Consortium

There are many factors affecting the pesticide remediation of bacteria-microbial consortium. The toxicity of the pesticide to members of the consortium, concentration, the site of contamination, temperature, pH, sunlight, and water availability all affect how a microbial consortium will accumulate or degrade a pesticide pollutant (Fang et al. 2010; Zhang et al. 2012). Furthermore, it is important to understand how the autochthonous organisms will interact with any inoculated organisms to avoid predation and competition (Cycoń et al. 2017).

Many pesticide pollutants may be toxic to the bacteria or algae species being used for remediation. Therefore, the concentration of the contaminant should be taken into consideration when designing the remediation. An example of this involves the uptake and transformation of the organophosphate nematocide fenamiphos into metabolites that were more toxic than the original compound (Cáceres et al. 2008a). While some species of *Chlorella* and *Anabaena* were able to oxidize fenamiphos, the oxidized by-product fenamiphos sulfoxide and bacterial metabolites fenamiphos phenol, fenamiphos sulfoxide phenol, and fenamiphos sulfone phenol were more toxic to *Pseudokirchneriella subcapitata* and *Chlorococcum* sp. (Cáceres et al. 2008a, b). Therefore, the microbial consortium should be designed to handle the uptake and transformation of all metabolic by-products of each species in the consortium. While these algae wouldn't be able to fully mineralize fenamiphos due to the toxicity of its transformation metabolite, pairing it with a bacterial species capable of degrading the partially oxidized phenols may prove effective, including strains from *Microbacterium*, *Sinorhizobium*, *Brevundimonas*, *Ralstonia*, and *Cupriavidus* genera (Cabrera et al. 2010). The wide variety of organisms capable of degradation have immense potential for combining bacteria and algae in bioremediation attempts.

The pH of soils has been shown to influence the degradation capabilities of some microbial consortiums. One study observed that the degradation of fenamiphos was improved as the pH of that soil increases, the more successful pHs being between 7.7 and 8.4 (Singh et al. 2003). It is hypothesized that more alkaline soils allow for higher expression of enzymes and higher total biomass (Singh et al. 2003). This may be exploited during ex situ bioremediation or bioaugmentation to enhance the efficacy of degradation, while changing the pH of a large contamination site may not be feasible. However, many strains of cyanobacteria and algae have been shown to produce extra polymeric substrates, sugars, proteins, and lipids under alkaline conditions which can enhance the growth and proliferation of heterotrophic bacteria (Subashchandrabose et al. 2011). This is just one example of the synergistic potentials of bacterial-algal consortiums. Another important factor affecting the bioremediation potentials is temperature.

Zhang et al. (2012) observed that γ -HCH (Lindane) was degraded faster at higher temperatures where 67.3% was degraded at 35 °C and 56.2% at 30 °C (Zhang et al. 2012). Fang et al. (2010) showed that DDT and associated metabolites were optimally degraded at 30 °C compared to 20 and 40 °C (Fang et al. 2010). This parameter would be nearly impossible to control in large in situ remediation attempts but should be considered when deciding on species consortiums and application types.

6.6 Limitations of Bacterial-Microalgal Consortium

One of the main limitations to the applied remediation consortium involves the specificity of strain to pollutant, as some strains of algae respond to different pesticides differently even resulting in toxicity (Subashchandrabose et al. 2011). This is not a one-size-fits-all solution. There is still a modicum of investigation needed to carry out effective remediation. Furthermore, some algal species are too sensitive to the toxicity of certain pesticides such as diazinon (Tien et al. 2011). This chapter was unable to find any species of microalgae capable of degrading or withstanding dieldrin or glyphosate.

The stability of a constructed consortium is only effective if there is division of labor and effective chemical communication between the species (Subash chandra bose et al. 2011). Inter-kingdom quorum sensing controls biofilm formation, co-metabolism, and stress responses but may not be compatible among some algae and bacteria. Furthermore, some nutrient requirements and physiochemical needs may not be compatible within every consortium. It is also hard to predict remediation outcomes based on laboratory and small pilot scale studies. The scalability of algal-bacteria consortiums may be a hindering aspect of its use and application.

In some instances, the metabolites produced during a biodegradation are more toxic than their precursors. For instance, one study observed that *Chlorella vulgaris* mediate degradation of diclofop-methyl (DM) to a less toxic metabolite diclofop (DC) (Cai et al. 2009). However, DC was then further degraded to 4-(2,4-dichlorophenoxy) phenol (DP) which was the most toxic metabolite of the

three intermediates (Cai et al. 2009; Subashchandrabose et al. 2013). This was also discussed earlier in the case of fenamiphos by-products of bacterial metabolism being toxic to certain algal species. These are potential setbacks to applied remediation attempts due to the production of metabolites more harmful than the pesticide which was originally contaminated. The metabolites of all components of the microbial community should be taken into account during the engineering of a bacterial-algal consortium.

Not all algae-bacteria interactions are commensal or mutualistic, which is vital to engineering effective consortiums. These include quorum sensing inhibitors, algicidal metabolites produced by bacteria, and limiting nutrient competition (Amin et al. 2012). However, by identifying already present microbes in polluted areas, these types of interactions can be avoided during the remediation application.

7 Synergistic Potentials of Combined Remediation

Algae-bacteria relations have been studied heavily for decades primarily focusing on symbiosis of nutrient exchange, chelation, bacterial attachment, co-metabolism, and chemical communication (Rengifo-Gallego and Salamanca 2015; Subashchandrabose et al. 2011). Much of the symbiosis between microalgae and bacteria is based on nutrient exchange of vitamins, iron, and fixed nitrogen (Cooper and Smith 2015; Ramanan et al. 2016). In one example, some algae lacking a methionine synthase gene cannot produce vitamin B₁₂ and require an exogenous source, which is produced by mutualistic bacteria species who in turn benefit from organic matter produced by the algae (Amin et al. 2012). Co-cultures of algae and bacteria have been shown to be more robust in the event of environmental flux and provide resistance to outside invasion or competition (Subashchandrabose et al. 2011). Furthermore, in the post omics age, mutualisms are being defined more closely and are providing insight into the application potentials of the mutualistic organisms (Cooper and Smith 2015). Biofilms conferring mutual advantage are described in Fig. 1. The relationship between archaea and microalgae is less well-known, but there is significant evidence for the interaction between the two kingdom based on chemical markers in marine sediment (Amin et al. 2012).

The relationship between the two kingdoms provides a unique opportunity to exploit the mutualism and synergy developed over millennia for the application of polymicrobial consortium for the bioremediation of pesticides. More comparative studies are needed to elucidate these relationships. Commonly, microbial-algal symbiosis occurs through the formation of biofilm. This association of consortiums through a biofilm matrix enhances the mutualisms previously discussed by bringing microbial populations closer together. This spatial organization makes for more efficient chemical communication, accumulation of limiting metals, and nutrient exchange. It has been shown that the heterotrophic bacteria have high O₂ demand during degradation of complex organic molecules and therefore thrive in the upper layers of cyanobacteria and algal mats (Abed 2010). Furthermore, some cyanobac-

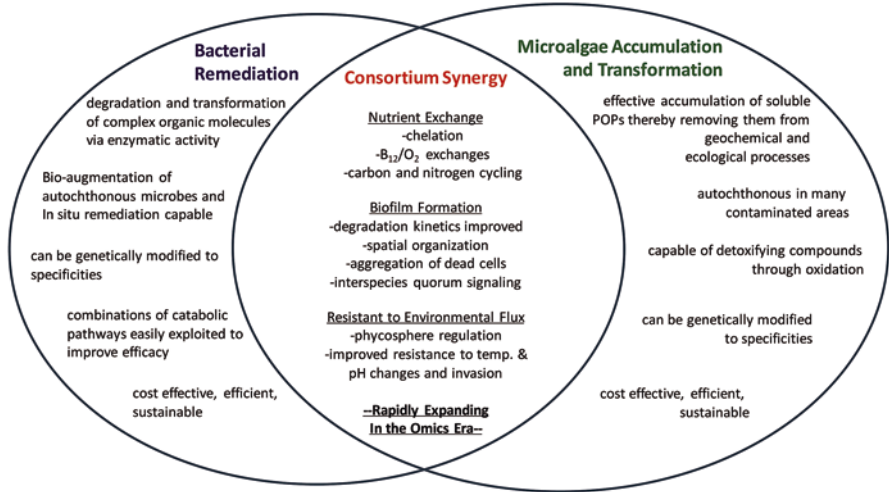


Fig. 1 Description of the benefits of biofilm formation between microalgae and bacteria. Various genera of bacteria and microalgae from selected studies included

teria even seem to regulate the eubacteria associated within their phycosphere by releasing certain carbon sources utilized by said eubacteria (Amin et al. 2012).

Biofilm formation may improve the remediation potentials of microalgal-bacterial consortiums. One study found that by immobilizing cells, they were able to increase the efficiency of the biodegradation of a polymicrobial consortium. This methodology immobilized cells on alginate beads, similar to the naturally occurring biofilms, which in turn improved the catalytic activity of the enzymes which increased the degradation efficiency (Yañez-Ocampo et al. 2009). This is further supported by an investigation into how biofilm formation improves the kinetics of degradation of diazinon, an organophosphate insecticide. It was shown that diazinon removal was 99.9% by algal-bacterial consortiums in biofilm compared to only 27% removal by the same species not in biofilm (Tien et al. 2011). This change in remediation was likely caused by the increased biomass associated with naturally occurring biofilms. This study also suggested that the remediation of diazinon was more efficient in springtime biofilms (99.9% removal) than wintertime biofilms (77% removal) due to higher levels of cellular absorption and adsorptions by extracellular polymeric substrate as well as increased levels of microbial degradation (Tien et al. 2011). Furthermore, cell aggregation and attachment will yield population increases in bacteria as algal cells die and begin to decompose. These dead and still attached algal cells provide alternative carbon sources thereby increasing bacterial populations, which has been shown to improve degradation potentials (Cycoń et al. 2017; Pino and Peñuela 2011).

The overall degradation of bacterial consortiums can be improved through the addition of an additional carbon source. For instance, one study using a fairly diverse consortium (Fig. 2) improved the degradation efficiency of methyl parathion by 28% and chlorpyrifos by 64% just by adding glucose to the medium (Pino and

Peñuela 2011). They reasoned that this additional carbon source greatly enhances the number of organisms in media thereby radically improving the overall degradation. One of the benefits reaped by bacteria through an algal mutualism is the addition of alternative carbon sources from the microalgae, thus further improving the remediation potential through increasing the number of bacteria.

The pH requirement of microbial consortiums can be matched and even manipulated to improve the degradation of pesticide pollutants. Fenamiphos degradation by bacteria was enhanced in alkaline soils, while *Scenedesmus*, *Chlamydomonas*, *Stichococcus*, *Chlorella*, *Nostoc*, and *Anabaena* species were all shown to accumulate and partially oxidize the same compound (Singh et al. 2003). Furthermore, bacterial degradation increased with the pH, while all the previous microalgae species are known to produce extracellular polymeric substrates, sugars, lipids, and vitamins that can be used as growth substrates by bacteria (Singh et al. 2003; Subashchandrabose et al. 2011). These interactions are described in Fig. 2.

8 Future Prospects

The future potentials of using a microalgae-bacterial consortium revolve around mitigating the limitations and can be improved upon in different ways. One such improvement includes the detection and selection of algal strains that are capable of withstanding larger environmental variation. In finding strains capable of growing at higher or lower pH, temperature, light availability, and pesticide levels, consortiums can be used in a wider range of applications. The toxicity of pesticides to

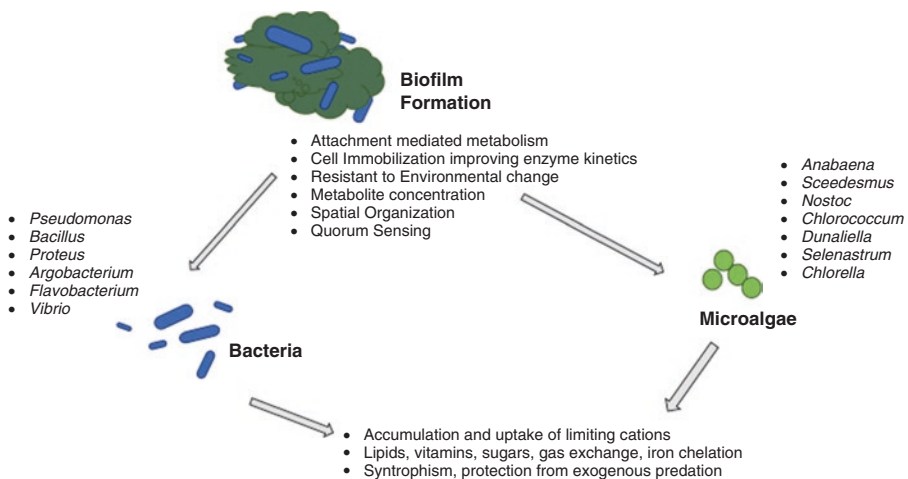


Fig. 2 Synergistic potentials of a bacterial-microalgal consortium to the application of bioremediation of pesticides

certain strains of algae remains a limitation in the application of microalgae-bacterial consortiums and may be improved by finding strains in extreme pollution settings.

Another future potential of engineering microalgae-bacterial consortium revolves around the long-term maintenance of homeostasis between species (Brenner et al. 2008). Engineering strains that can be selected for or against to control consortium ratios can be a potential fix. Finding ways to maintain the balance of organisms to optimize the remediation could greatly benefit from further study. Using alternative carbon sources and/or differential antibiotic regimes could be ways to control these populations.

Bacterial consortium engineering can exploit external chemical cues, like isopropyl β -D-1-thiogalactopyranoside (IPTG), to induce genetic circuits to promote commensalism and cooperation (Brenner et al. 2008). Inducible circuits should be further investigated to apply this concept to microalgae-bacterial populations. By elucidating the mechanisms that can confer interspecies communication and quorum sensing, these microalgae-bacterial consortiums can be tightly regulated with outside signals.

Auxotrophic mutants can be generated and used to make two species completely reliant upon one another. Research should be directed in ways to expand the ability to remediate pesticides that may be toxic to one or more members of the consortium and to control and augment the consortium ratios to improve the efficiency of remediation. Constructing consortiums that collapse when one or more members of the consortium expire can mitigate any ecological effects or imbalances associated with in situ remediation.

More research into the biochemical pathways involving the catabolism of consortiums would allow for more efficient remediation and novel applications. New “omics” tools and computational systems approaches can be employed for the development of consortium-based remediation.

Nanotechnologies including nano-adsorbents, nano-membrane-based filtration, and nanoparticle catalysts can be used in every stage of a remediation pipeline to improve a wide variety of processes (Gaur et al. 2018).

One study used a microalgae-bacterial consortium to anaerobically digest the microalgal biomass to produce methane for use as a biofuel. Using activated sludge from a wastewater treatment plant in Spain, they observed that low phosphorus levels of the incoming wastewater led to increases in lipids found in algal biomass (Hernández et al. 2013). More research can be done to improve the collection of biomasses to be used in fertilizers, pigments, animal feed, and nutrition supplements (Spolaore et al. 2006; Subashchandrabose et al. 2011). These future directions are described in Fig. 3 with various areas of research showing potential improvements in many places among the bioremediation pipeline. The general pipeline is described, and the areas of future improvement are demarcated with a yellow lightning strike and include nano-based technologies, consortium engineering, in situ discovery of novel strains, and improvements in the usage of incidental algal biomass in industrial applications.

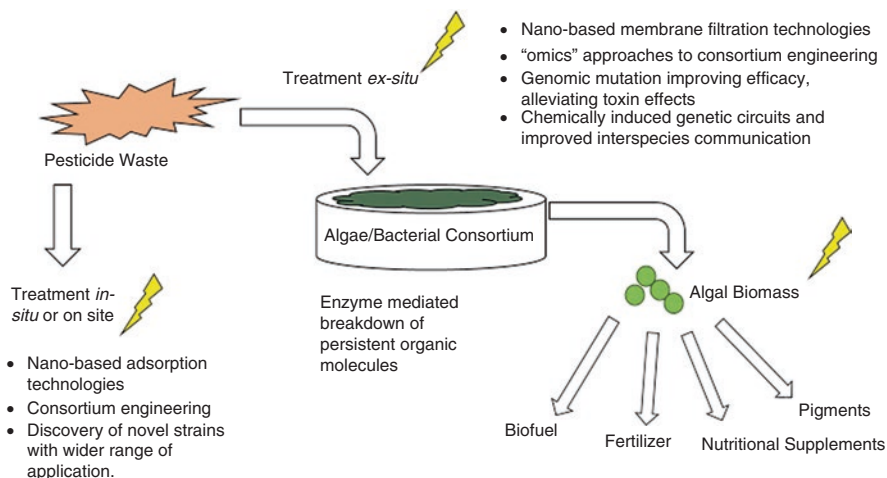


Fig. 3 Future potentials in the improvement of the bioremediation of pesticides using a microalgae-bacterial consortium

9 Conclusion

The use of pesticides has evolved and steadily increased since their first uses in the middle of last century. The production and application of pesticides is justified through overall increases in crop production and protection as well as through control of disease vector pests. The application of pesticides can be harmful to those involved and often leads to their deposit in aquatic and soil environments where many of them are resistant to degradation and accumulate over time. They may even enter the food web through primary producers becoming slowly magnified eventually becoming a risk to human health. The current levels of pesticides in the environment, water supply, and food stuffs have led to the need for developing efficient and cost-effective methods for their remediation and removal from these ecosystems. Bioremediation has been developed and used for these very reasons, and the bacterial-microalgae consortium applications were discussed in this chapter. By combining bacterial degradation with the bioaccumulation and degradation potentials of microalgae and cyanobacteria, the overall efficacy of bioremediation is improved. This is dependent upon endogenous characteristics of the consortium as well as the physiochemical aspects of the polluted site. While there are many limitations to the application of the bacterial-microalgal consortium, it remains wholly feasible and easily exploitable while ripe for further study and analysis. With over 200 million years of coevolution, these microbial consortiums can be used as an effective tool for the bioremediation and bioaugmentation of pesticide pollution (Table 1).

Table 1 Potential consortiums based on the degradation potentials of bacteria and algae by pesticide and type

Pesticide	Bacteria	References	Algae	References
HCH (Lindane)	<i>Pseudomonas</i> , <i>Burkholderia</i> , <i>Flavobacterium</i> , and <i>Vibrio</i>	Velázquez-Fernández et al. (2012).	<i>Anabaena azotica</i> <i>Scenedesmus intermedius</i>	Zhang et al. (2012) González et al. (2012).
DDT	<i>Pseudomonas</i> , <i>Neisseria</i> , <i>Moraxella</i> , and <i>Acinetobacter</i>	Velázquez-Fernández et al. (2012)	<i>Chlorococcum</i> sp., <i>Anabaena</i> sp., <i>Nostoc</i> sp., <i>Aulosira fertilissima</i>	Subashchandrabose et al. (2013)
	<i>Sphingobacterium</i>	Fang et al. (2010)		
Endosulfan	<i>Pseudomonas spinosa</i> , <i>Pseudomonas aeruginosa</i> , and <i>Burkholderia</i>	Velázquez-Fernández et al. (2012)	<i>Chlorococcum</i> sp., <i>Scenedesmus</i> sp.	Sethunathan et al. (2004)
Dieldrin and endrin	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Trichoderma</i> , <i>Aerobacter</i> , <i>Mucor</i> , <i>Micrococcus</i> , and <i>Burkholderia</i>	Velázquez-Fernández et al. (2012)	<i>Dunaliella</i> sp. and <i>Agmenellum quadruplicatum</i> ^a	Matsumoto et al. (2009); Patil et al. (1972)
Atrazine	<i>Bacillus</i> , <i>Pseudomonas</i> , and <i>Burkholderia</i>	Dutta et al. (2016)	<i>Ankistrodesmus</i> sp. <i>Selenastrum</i> sp.	Geed et al. (2017)
Dimethomorph/pyrimethanil	-	-	<i>Scenedesmus obliquus</i> , <i>Scenedesmus quadricauda</i>	Dosnon-Olette et al. (2010)
Glyphosate	<i>Pseudomonas</i> spp. strains GA07, GA09, and GC04	Zhao et al. (2015)	<i>Isochrysis galbana</i> , <i>Emiliania huxleyi</i> , <i>Skeletonema costatum</i> , <i>Phaeodactylum tricornutum</i>	Wang et al. (2016)
Methyl parathion	<i>Agrobacterium radiobacter</i> ; <i>Burkholderia</i> (<i>Pseudomonas</i>) <i>pseudomallei</i> , <i>Sinorhizobium meliloti</i> , <i>Ochrobactrum anthropi</i>	Hove-Jensen et al. (2014)	<i>Leptolyngbya boryana</i> , <i>Microcystis aeruginosa</i> , and <i>Nostoc punctiforme</i>	Hove-Jensen et al. (2014)
	<i>Proteus vulgaris</i> , <i>Acinetobacter</i> sp., <i>Flavobacterium</i> sp., <i>Pseudomonas putida</i> , <i>Citrobacter freundii</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus</i> sp., <i>Stenotrophomonas</i> sp., <i>Pseudomonas</i> sp., and <i>Proteus</i> sp.	Pino and Peñuela (2011)	<i>P. foveolarum</i> , <i>O. animalis</i> , <i>N. muscorum</i> , <i>N. linckia</i> , <i>S. bijugatus</i> , <i>C. vulgaris</i>	Megharaj et al. (1994)

continued

Table 1 continued

Pesticide	Bacteria	References	Algae	References
Fenamiphos	<i>Brevibacterium</i> sp.	Cáceres et al. (2008a)	<i>P. subcapitata</i> , <i>Chlorococcum</i> sp.	Cáceres et al. (2008b)
	<i>Microbacterium</i> , <i>Sinorhizobium</i> , <i>Brevundimonas</i> , <i>Ralstonia</i> , <i>Cupriavidus</i>	Cabrera et al. (2010)	<i>Scenedesmus</i> sp. <i>Chlamydomonas</i> sp. <i>Stichococcus</i> sp. <i>Chlorella</i> sp. <i>Nostoc</i> sp.	
Prometryne	<i>Ochrobactrum</i> sp., <i>Bacillus</i> sp. degradation was greatly enhanced by inoculation with nematodes	Zhou et al. (2012)	<i>Chlamydomonas reinhardtii</i>	Jin et al. (2012)
Fluroxypyr	Unknown consortium of soil bacteria	Tao and Yang (2011)	<i>Chlamydomonas reinhardtii</i>	Jin et al. (2012)

^a*Dunaliella* sp. and *Agmenellum quadruplicatum* were shown to metabolize aldrin and dieldrin, but further study is suggested as the by-products; photodieldrin and ketoendrin are more toxic than the parent compounds

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