Part 1

MICROBIAL BIOREMEDIATION AND BIOPOLYMER TECHNOLOGY

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A Recent Perspective on Bioremediation of Agrochemicals by Microalgae: Aspects and Strategies

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Abstract

With the increasing world's population, enhancement of crop production has become a major target for mankind survival. This leads to extensive use of agrochemicals which has revolutionized the entire pest control system. However, due to their uncontrolled use, the equilibrium between their beneficial effects and harmful consequences has been compromised which lead to severe environmental havoc. To combat their hazardous influences, several remediation methods such as adsorption and ultrasonic irradiation have been developed. But unfortunately, most of them are not cost-effective and environment-friendly. As a result, bioremediation has become a potential alternative to these remediation methods being less expensive and eco-friendly. Microalgae have recently received sufficient attention as a bioremediation candidate due to their cheap nutritional requirements (solar light and CO_2) and versatile metabolic activities. The microalgae-based remediation technologies are ecologically more comprehensive and can be integrated with several other technologies such as biofuel production and carbon mitigation. Regardless of these conveniences, a critical scrutiny of the current status of the technology is required to get an in-depth insight into the applicability of microalgae for remediation of pollutants. The present article is an attempt to provide a crucial look into the microalgae-based removal of agricultural pollutants and an outline of its mechanistic perspectives. Also, molecular aspects of bioremediation by microalgae have been discussed to provide a better understanding of its remediation capabilities.

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1.1 Introduction

The human population is constantly increasing at a fast rate and might reach around 9.7 billion people by 2050 [1]. To satisfy the food requirements of this enormous population, an enhancement in crop production is needed which open doors for the use of various agrochemicals. Agrochemicals (fertilizers and pesticides) are the group of chemicals used in agricultural practices to improve crop yield. Limited availability of macronutrients such as nitrogen, phosphorus, and potassium may result in poor growth of the crop. Thus, commercial fertilizers enriched with these ingredients may be applied to meet the demand of the essential elements. But plants can absorb only a limited amount of these nutrients and the excess fertilizer may be washed down along with the rain into water bodies and thereby causing contamination of the same [2].

Thus, uncontrolled and excessive use of fertilizers (e.g., phosphate fertilizer) may result in eutrophication of canals and reservoirs [3, 4]. In addition to chemical fertilizers, another chemical extensively used in agricultural activities is pesticides. These substances are utilized to control the infestations by crop destroying organisms referred to as pest and thereby enhancing agricultural productivity [5]. Although these agrochemicals are used to benefit humans, they have a hazardous impact on the environment [6]. Thus, with the use of several million tons of agrochemicals every year, the agricultural sector has been considered to be a major source of environmental pollution [7].

Among the various agrochemicals used in modern agricultural practice, pesticide and its residues pose a serious threat to environmental health and stability [8]. As a result, environmental pollution due to pesticide has become a major global concern. Pesticides can be defined as substances (or a mixture of substances) developed to repel or mitigate pests [9]. Pesticides include a wide array of compounds intended to reduce crop destroying agents such as insects, weeds, fungi, and rodents. These pesticides vary in their physical as well as chemical properties, and hence, it is important to classify them which make their study convenient. Although there are various ways of pesticide classification, the one based on their chemical composition are the most used one. This type of classification provides a proper correlation between structural features, activity, toxicity, and degradation mechanisms, among different members [5]. Based on chemical composition, pesticides have been classified into four major classes, namely, organochlorines, organophosphorus, carbamates, and pyrethroids [10]. Table 1.1 shows the chemical composition and general characteristics of important pesticides [11, 12].

Table 1.1 Chemical composition and general characteristics of different pesticide groups [11, 12].

Group	Chemical Composition	General Characteristics	Example
Organochlorines	Composed of C, H, Cl, and sometimes "O" is also present.	Lipid soluble, accumulation in fat rich animal tissue, persistent for a longer period, nonpolar in nature.	Lindane, endosulfan, mirex, DDT
Organophosphate	Phosphorus atom occupies central position within the molecule. They may be heterocyclic, cyclic, and aliphatic.	Shows solubility in water and organic solvents, low persistence compared to organochlorines, the central nervous system gets affected by these compounds.	Diazinon, methyl parathion, malathion
Carbamates	Chemical structure is similar to a plant alkaloid produced by *Physostigma venenosum*.	Derived from carbamate acid; have high vertebrate toxicity; less persistent.	Carbaryl, sevin
Pyrethroids	Chemical structure is based on pyrethrin obtained from *Chrysanthemum cinerariifolium*.	Affect the nervous system; are less persistent compared to other pesticides.	Pyrethrins

Although pesticides benefit human by enhancing agricultural productivity, they adversely affect human beings as well as other non-target organisms (explained in details in Section 1.2). Thus, remediation of these anthropogenic compounds is highly essential. Scientists have developed several physical and chemical remediation methods such as adsorption, oxidation, ozonation, nanofiltration, and membrane filtration ultrasonic irradiation for pesticide elimination from the environmental matrices and thereby minimizing their hazardous influences [13, 14]. But unfortunately, most of these methods are not environment-friendly and the cost associated with them is very high. As such, there is a requirement of an alternative technology devoid of these limitations. Bioremediation being inexpensive and eco-friendly proves itself as a potential replacement to various physical and chemical remediation methods. Earlier researchers have focused mainly on bioremediation using fungal and bacterial strains [15]. But recently, microalgae have received sufficient attention as an efficient bioremediation candidate due to their versatile metabolic activities, low-cost nutritional requirements (solar light and CO_2), and ability to survive in different environmental conditions [13]. The aim of this article is to summarize and evaluate the various aspects of bioremediation of pesticides using microalgae with attention on microalgal species involved, strategies, molecular basis, and factor affecting the process.

1.2 Pollution Due to Pesticides

Pesticides are anthropogenic compounds developed for human welfare by improving agricultural productivity. The estimated loss of agricultural products is 40% worldwide due to the effect of various agents such as plant diseases, pests, and weeds. Accordingly, the utilization of pesticide in agriculture has counteracted increment in this rate [6]. This is a roundabout way lessens the likelihood of price rise due to the decline in food production as a consequence of low agricultural productivity.

In addition to crop protection, pesticides also contribute to human health improvement by killing insect and rodent vectors responsible for spreading diseases. Pesticide application has been found useful in controlling various diseases such as typhus, bubonic plague, encephalitis, typhoid fever, and yellow fever, which are mainly vector-borne [16, 17]. Despite these beneficial effects, pesticides have several harmful consequences which outshadow its beneficial impacts.

Depending on solubility, pesticides can get entry into the ecosystem mainly by two processes: firstly, pesticides which are water soluble directly enter the water bodies such as ponds, rivers, lakes, and streams by getting dissolved in water and thereby adversely affecting the non-target life forms. Secondly, fat soluble pesticides get dissolved in the tissues of animals and move from one trophic level to the next through the food chain. The concentration of the pesticides in each trophic level increases as it passes from one trophic level to the other by the process of bioamplification [18] (Figure 1.1).

Pesticides drifting from land into various water bodies such as rivers and lakes adversely affect the aquatic ecosystem. Aquatic plants are an important component of the aquatic ecosystem and are responsible for providing approximately 80% of the dissolved oxygen [6]. Death of plants due to pesticides (e.g., herbicide) can lower the level of O_2 and aquatic organisms such as fishes can suffer due to oxygen depletion. This may further result in a reduction in fish productivity [19]. In addition to fishes, amphibian species are also affected by pesticide exposure. For instance, Rohr *et al.* [20] demonstrated a toxic impact of herbicide atrazine on some fish and amphibian species. Their mesocosm study revealed a relationship between exposure of herbicide atrazine and abundance alteration of larval trematodes in northern leopard frogs.

In addition to the aquatic ecosystem, terrestrial ecosystems are also adversely affected by the uncontrolled use of pesticide. Both target and non-target plants are affected by pesticide application. For instance, disease susceptibility of plants can be accelerated due to the application of herbicide glyphosate [21]. Further, the yield of non-targeted crops can be adversely affected by herbicides; sulphonamides, sulfonylureas, and imidazolinones [22]. Excessive use of pesticides also has deleterious effects on beneficial microbes present in the soil. Many soil dwelling microbes are

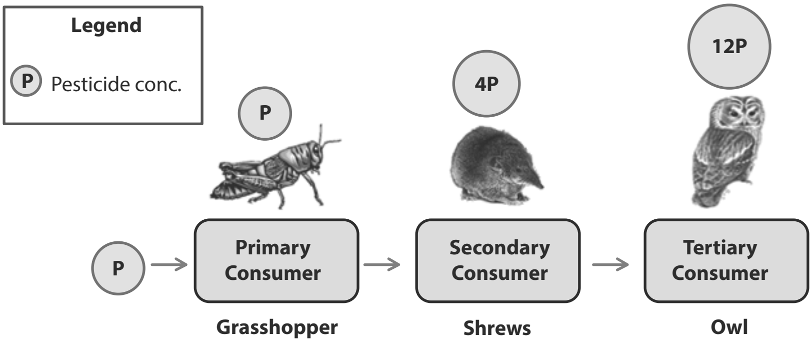

Figure 1.1 A diagrammatic representation of pesticide bioamplification in the environment [45].

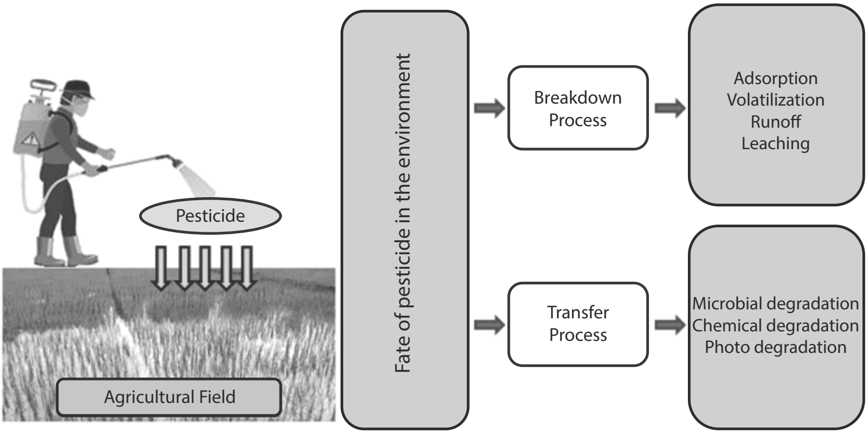

Figure 1.2 A diagrammatic representation of fate of pesticide in the environment [35].

involved in atmospheric nitrogen fixation. Pesticide can have a dangerous impact on these microbial communities. For example, growth and activity of soil dwelling bacteria can be negatively influenced by glyphosate [23]. Furthermore, nitrification and denitrification processes can be drastically altered by chlorothalonil and dinitrophenyl fungicides [24] (Figure 1.2).

Although pesticides contribute to the improvement of human health by controlling disease causing vectors (as mentioned earlier), it has several adverse effects as well. World Health Organization (WHO) states that about 30 lakhs cases of pesticide poisoning and 2 lakhs 20 thousand cases of death is reported annually in developing countries [25, 29]. In addition, 22 lakhs people are in danger of adverse pesticide impact in these nations [26]. Pesticides invade living system by three major routes: ingestion, inhalation, and dermal penetration [27]. Inspite of body's capacity to degrade and excrete pesticides, some residues may occur in the system due to absorption by the blood [28]. This may result in both acute and chronic adverse effects in humans. Infants, children, pesticide applicators, and those working in agricultural farms are considered to the main victims of the adverse impact of pesticides [29].

1.2.1 Acute Effects

Effects that occur after immediate exposure to pesticides are referred to as acute effects. These effects include skin itching, an occurrence of skin blisters and rashes, nose and throat irritation, blurred vision, vomiting and nausea, and diarrhoea. Acute effects are not serious enough to seek medical help and are rarely fatal [29].

1.2.2 Chronic Effects

Chronic effects of pesticides refer to its long term effects which may require even years to appear. Various body organs such as the lungs, liver, and kidney may be adversely affected due to the chronic impact of pesticides [6]. Reduction in motor signalling and visual ability, as well as impaired coordination and memory, can be attributed to the chronic effects of pesticide exposure [25]. Alteration in levels of human reproductive hormones (male and female) due to prolong presence of pesticide in the body may adversely affect reproductive potential and may result in infertility, stillbirth, birth defects, and spontaneous abortion [29]. Prolong exposure to pesticide may negatively affect the immune system and at the same time may cause various ailments such as hypersensitivity, asthma, and allergies [30]. Furthermore, various negative consequences such as nervousness, dizziness, confusion, nausea, vomiting, tremors, and hypersensitivity toward sound, light, and touch may occur due to ingestion of pesticides such as organochlorines [25].

1.3 Microalgal Species Involved in Bioremediation of Pesticides

Agrochemicals find widespread application in modern day agricultural practices to control pests and weeds to accelerate crop productivity. But environmental deterioration created by these chemicals has compelled human beings to look for an eco-friendly technology such as bioremediation. With the establishment of microalgae as an ideal bioremediation candidate, isolation and selection of strains which are resistant as well as have biodegrading potential received sufficient scientific attention. There are number of scientific investigations which reveal the pesticide degradation capabilities of cyanobacteria and algae (Table 1.2). According to Megharaj *et al.* [31] cyanobacteria *Nostoc linckia*, *Phormidium tenue*, and *Synechococcus elongatus* and green algae *Scenedesmus bijugatus* and *Chlorella vulgaris* had the capability to metabolise two organophosphorus insecticide monocrotophos and quinalphos. They concluded that both cyanobacteria and algae had similar biodegradation potential. In another work Megharaj *et al.* [32] also showed the biodegradation of the pesticide methyl parathion (MP)

Table 1.2 Cyanobacterial/microalgal strains involved in biodegradation of pesticide.

Chemical	**Microalgae/Cyanobacteria**	**Reference**
Monocrotophos and Quinalphos	*Chlorella vulgaris*, *Scenedesmus bijugatus*, *Synechococcus elongatus*, *Phormidium tenue*, *Nostoc linckia*	[31]
Methyl parathion	*C. vulgaris*, *S. bijugatus*, *N. linckia*, *N. muscorum*, *Oscillatoria animalis*, *P. foveolarum*	[32]
DDT	*Chlorococcum* sp., *Anabaena* sp., *Nostoc* sp.	[77]
α-Endosulfan	*Scenedesmus* sp., *Chlorococcum* sp.,	[76]
Fenamiphos	*Pseudokirchneriella subcapitata*, *Chlorococcum* sp.	[33]
Dimethomorph and Pyrimethanil	S. quadricauda	[39]
Fluroxypyr	Chlamydomonas reinhardtii	[40]
Chlorpyrifos	*Synechocystis* sp. strain PUPCCC 64	[41]
Prometryne	*C. reinhardtii*	[43]
Anilofos	*Synechocystis* sp. strain PUPCCC 64	[42]
Acephate, Imidaclorpid	*C. mexicana*	[44]
Diazinon	*C. vulgaris*	[13]
Methyl parathion	*Fischerella* sp.	[45]

by cyanobacteria *P. foveolarum*, *N. muscorum*, *N. linckia*, and *Oscillatoria animalis* and green algae *S. bijugatus* and *C. vulgaris*. The study showed that they were capable of hydrolyzing the insecticide in 20 days while *C. vulgaris*, *N. linckia*, and *S. bijugatus* could hydrolyze the same in 30 days. Thus, it concluded that the biodegradation capabilities of selected microalgal and cyanobacterial strain followed the following order: *C. vulgaris* < *S. bijugatus* < *N. linckia* < *N. muscorum* < *O. animalis* < *P. foveolarum*.

In addition to this, five green algae (*Chlorella* sp., *Scenedesmus* sp. MM1, *Stichococcus* sp., *Scenedesmus* sp. MM2, and *Chlamydomonas* sp.) and five cyanobacteria (*Anabaena* sp., *Nostoc* sp. MM1, *N. muscorum*, *Nostoc* sp. MM3, and *Nostoc* sp. MM2) have been reported to degrade fenamiphos which is an organophosphorus pesticide [33].

2,4-dichlorophenol (2,4-DCP) is often used as an intermediate in synthesis of insecticides and herbicides such as 2,4-D. Thus, the release of chlorophenols as industrial waste or by degradation of chlorinated pesticides have cause serious environmental threat [34]. Yang *et al.* [35] reported biotransformation and enzymatic responses of 2,4-dichlorophenol in *Skeletonema costatum* (diatom). They demonstrated that Cytochrome P-450, a key enzyme in biotransformation and metabolization, did not play an important role in 2,4-DCP detoxification.

Popular pest control agents such as chlorinated agrochemicals cause serious environmental problems such as accumulation in non-target organisms as well as in water and soil. Considering the high persistence and toxicity of chlorinated pesticide like lindane, many countries have prohibited its direct application [36]. Thus, there is a requirement of potential microalgal strain for eco-friendly remediation of chlorinated pesticides. Kuritz and Wolk [37] evaluated the lindane degrading potential of cyanobacteria *N. ellipsosporum* and *Anabaena* sp. genetically manipulated to biodegrade another contaminant 4-chlorobenzoate. Biodegradation of the pesticide lindane by the cyanobacterial strains *Synechococcus* sp., *Oscillatoria* sp., *Cyanothece* sp., *Nodularia* sp., *Synechococcus* sp., *Nostoc* sp., *Microcystis aeruginosa*, *A. cylindrical*, *M. aeruginosa*, *A. spiroides*, and *A. flos-aquae* has been reported [38].

Dosnon-Olette [39] demonstrated the removal of fungicides dimethomorph and pyrimethanil and herbicide isoproturon by the microalgae *S. quadricauda* and *S. obliquus*. The study showed that *S. quadricauda* removed dimethomorph and pyrimethanil more effectively than *S. obliquus*. Fluroxypyr (pesticide) accumulation and degradation by green alga *C. reinhardtii* was reported by Zhang [40]. They noted that *C. reinhardtii* had the potential to degrade more than 57% of bioaccumulated fluroxypyr within 5 days.

Singh *et al.* [41] demonstrated the potential of the cyanobacterium *Synechocystis* sp. to biodegrade the organophosphorus pesticide chlorpyrifos. The study showed that the organism could tolerate chlorpyrifos up to 15 mg L^{-1}. Maximum removal of chlorpyrifos was achieved at a temperature of 30°C, pH 7.0, and 100 mg protein^{-1} biomass. Metabolization of the pesticide by the cyanobacteria resulted in production of 3,5,6-trichloro-2-pyridinol as degradation product. The same cyanobacterial strain was later reported to degrade anilofos by Singh *et al.* [42]. In the study, the organism was found to tolerate high concentration of anilofos (25 mg L^{-1}).

The influenced of the pesticide on photosynthetic pigment content was dose-dependent. The herbicide was uptaken rapidly by the organism during the first 6 hours after which there was slow uptake until 5 days. The cyanobacterium utilized anilfos as a source of phosphate with maximum removal of anilofos at temperature of 30°C, pH 8.0, and 100 mg protein L^{-1}. In addition to cyanobacteria, microalgae are reported to degrade herbicides. For instance, the green alga *C. reinhardtii* was found to accumulate and biodegrade the pesticide prometryne. The study demonstrated that *C. reinhardtii* had the capacity to degrade prometryne at a moderate concentration of 5 g L^{-1}. This uptake and degradation of herbicide by *C. reinhardtii* reflect the internal tolerance mechanism of the green algae and establish it as a potential strain for remediation of prometryne from contaminated water [43].

In a recent study, Kurade *et al.* [13] found that *C. vulgaris* has the capacity of bioremediation of diazinon (Figure 1.3). In the study, the rate constant of degradation (k) of diazinon (0.5–100 mg L^{-1}) ranged between 0.2304 to 0.049 d^{-1} and the half-life ($T_{1/2}$) ranged between 3.01 and 14.06 d^{-1}. According to gas chromatography mass spectroscopic (GC-MS) study, metabolism of diazinon by microalgal strain resulted in the formation of 2-isopropyl-6-methyl-4-pyrimidinol (IMP) which is a by-product with low toxcity. In another work, Kumar *et al.* [44] studied the degradation of pesticide acephate and imidacloprid by the microalgae *C. mexicana*. They concluded that *C. mexicana* was able to remove 25% and 21% of acephate and imidacloprid, respectively. In another recent work, Tiwari *et al.* [45] demonstrated that cyanobacterium *Fischerella* sp. isolated from paddy

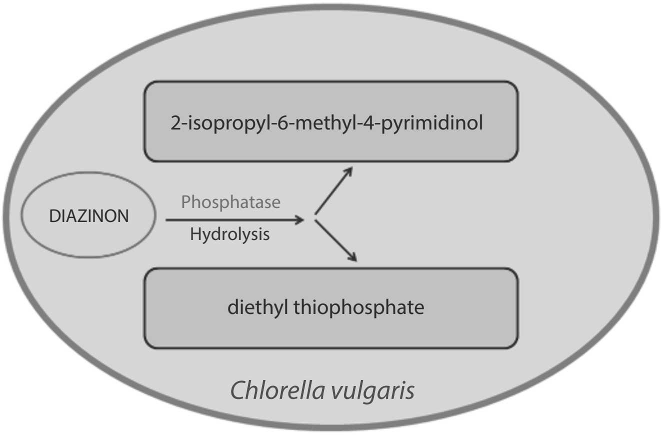

Figure 1.3 Schematic representation diazinon degradation by *Chlorella vulgaris* [38].

fields has the capacity to degrade organophosphorus pesticide MP. Based on their study, they recommend the organism as a potential candidate for pesticide bioremediation.

1.4 Strategies for Phycoremediation of Pesticides

1.4.1 Involvement of Enzymes in Phycoremediation of Pesticides

Biodegradation involves the breakdown of organic compounds into its inorganic constituents. Enzymes are one of the important biomolecules involved in the degradation of pesticides. The three main enzymes involved in pesticide degradation are hydrolases, esterases (also hydrolases), and the mixed function oxidases (MFOs). These enzyme systems are involved in the first metabolism stage of the pesticide and the glutathione S-transferase (GST) system, in the second phase [46]. In general pesticide, metabolism involves three main phases. During the Phase I of pesticide metabolism, the parent compound is converted into a more water-soluble and less toxic form by various processes such as oxidation, reduction, or hydrolysis. In the second phase, the water solubility and toxicity of the pesticide is further reduced by conjugation of the pesticide or pesticide metabolite to an amino acid or sugar. In the third phase, Phase II metabolites are converted into non-toxic secondary conjugates [46, 47]. Microalgae are photosynthetic organisms equipped with efficient enzyme system to metabolize and degrade various organic pollutants such as pesticides. Pertaining to their potential to degrade pesticides, microalgal species are recommended for remediation of the site contaminated with highly toxic pesticide like lindane [8]. Degradation of organophosphorus pesticide in presence of microbial enzymes has attracted the attention of scientist across the world. For instance, the enzyme alkaline phosphatase secreted by *Spirulina platensis* can hydrolyze chlorpyrifos, an organophosphorus pesticide, into 3,5,6-trichloro-2-pyridinol (TCP) [48]. Thus, immobilization of these pesticide degrading enzymes secreted form microalgae on solid matrix can be employed for remediation of pesticide contaminated sites [8].

1.4.2 Use of Genetically Engineered Microalgae

Development of genetically manipulated microalgae is a modern technology. This involves overexpression of contains proteins and enzymes which can combat the toxic effect of the contaminant. Extensive sequence information and good background knowledge about molecular, biochemical, physiological, and ecological characteristics of the microalgal species are required for the development of transgenic species to be used for bioremediation [49]. According to studies, *Anabaena* sp. strain PCC7120 and *N. ellipsossorum* are capable of degrading γ-Hexachlorocyclohexane (HCH) [37]. These two strains showed enhanced degradation of lindane when they are genetically modified using Lin A gene [37]. Thus, microorganisms can be genetically modified to develop highly efficient pesticide degradation strains which can be employed for eco-friendly remediation of pesticides.

1.5 Molecular Aspects of Pesticide Biodegradation by Microalgae

Several scientific studies are available in which algae and cyanobacteria have been reported to be highly efficient in detoxification of xenobiotics such as pesticides. Singh *et al.* [41] reported the degradation of the organophosphorus insecticide chlorpyrifos by the cyanobacterium *Synechocystis* PUPCCC. According to the author, the degradation mechanism of chlorpyrifos by cyanobacteria might be similar to bacteria. In bacteria, phosphotriesterases are the major group of enzymes involved in degradation of organophosphate pesticides [50]. These enzymes are encoded by a gene called *opd* (organophosphate-degrading). Mulbry and Karns [51] cloned and sequenced the gene. Phosphotriesterases are responsible for hydrolysis of phosphoester bonds, such as P–O, P–F, P–NC, and P–S [52]. The *opd* gene encoding organophosphorus hydrolase (the enzyme responsible for degradation of organophosphate pesticide) has 996 nucleotides, a typical promoter sequence of the promoter TTGCAA N17 TATACT from *E. coli* [53]. Chungjatupornchai and Fa-Aroonsawat [54] expressed opd gene from *Flavobacterium* sp. both on the surface and intracellularly in the cyanobacterium *Synechococcus* PCC7942 and used it for biodegradation of organophosphate pesticide. This reflects the importance of *opd* gene in biodegradation of organophosphate pesticides.

Exposure of plants to toxic organic substances provokes production of intracellular reactive oxygen species (ROS) which may adversely affect various cellular functions such as peroxidation of lipids and oxidation of proteins [55]. In order to minimize the adverse effects of ROS, plants possess an elaborate defense system consisting of antioxidant enzymes. Scavenging of ROS depends on the coordinated function of antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), and Ascorbate peroxidise (APX) [56]. SOD is involved in the dismutation of superoxide anion O_2^- to

H_2O_2 and O_2. H_2O_2 is further scavenged by the catalytic activity of CAT and APX. APX is involved in the ascorbate-glutathione cycle to reduce stress [55, 57]. Jin *et al.* [43] reported an upregulation of the genes encoding Mn-SOD, CAT, and APX in green alga *C. reinhardtii* exposed to the herbicide prometryne. An efficient scavenging/detoxification system is responsible for quick accumulation and degradation of pesticide by microalgae [40]. Jin *et al.* [43] also noted an upregulation of the inducible gene HO-1 (Heme Oxygenase-1) in *C. reinhardtii* exposed to the herbicide prometryne suggesting its involvement in the tolerance of the microalgae toward the herbicide. Kumari *et al.* [58] evaluated butachlor toxicity in *Aulosira fertilissima* using a proteomic approach. They concluded that out of eight proteins altered during butachlor exposure, downregulation of GroES (associated with protein folding), and overexpression of NusB (associated with transcription termination) are curtail for cell death. Molecular docking studies confirm that interaction of butachlor with GroES and NusB is responsible for its toxicity [59].

Agrawal *et al.* [60] demonstrated the molecular basis of butachlor toxicity/tolerance in three *Anabaena* species using comparative proteomics. The study showed that 75 proteins involved in photosynthesis, C, N and protein metabolism, redox homeostasis, and signal transduction were differentially expressed in each *Anabaena* sp. Agrawal *et al.* [61] reported that a novel aldo-keto reductase (AKR17A1) from *Anabaena* sp.7120 has the capacity to degrade chloroacetanilide herbicide butachlor. The study demonstrated that, in addition to combating multiple stresses, aldo-keto reductase encoding open reading frame all 2,316 plays a significant role in butachlor degradation. The gene can be used to develop transgenics with butachlor degradation and stress tolerance capabilities [61].

For evaluation of biodegradation and biotransformation of pesticide by microalgae, time-dependent environmental risk assessment is very essential [62]. Esperanza *et al.* [63] evaluated the toxicity of the widespread herbicide atrazine to the green alga *C. reinhardtii* by the transcriptomic and proteomic approach. They found that exposure of the microalgae to sublethal concentration of atrazine (0.25 μM) for 3 h resulted in differential expression of 185 genes, of this 124 showed upregulation and 61 genes showed downregulation. These genes belonged to 13 different categories of function such as photosynthesis, metabolism, gene expression, energy, amino acids, cell cycle, redox, lipid, regulation, ROS and stress, proteases, other and unknown [64]. They also noted that nine genes related to photosynthesis were differentially expressed, of which three genes (HLA3, LCIA, and ELI3) showed significant upregulation and six genes (LHCBM8, LHCSR3, LI818R-1, PTOX2, CAH4, and CAH5) showed significant downregulation. In a recent study Tiwari *et al.* [65] demonstrated the tolerance strategy of cyanobacteria *Fischerella* sp. exposed to organophosphorus insecticide MP by analyses of proteome and transcriptome. Proteome analysis revealed a differential expression of proteins connected to various metabolic activities such as photosynthesis, energy and protein metabolism, redox homeostasis, signal transduction, and cellular defense. Transcript analyses showed differential expression of genes such as phycocyanin α subunit (*cpcA*), ribulose bisphosphate carboxylase (*rbcl*), F0F1 ATP synthase subunit α, F0F1 ATP synthase subunit β, SOD (*sod*), NifH (nif H), DnaK (*dnaK*), and Peptidase S8 in *Fischerella* sp. exposed to MP. In addition, some hypothetical proteins related to signaling and carbohydrate metabolism were also found to be upregulated in the cyanobacterium exposed to MP stress. One hypothetical protein was found to be homologous to lectin with an MP binding pocket. The author suggests that this carbohydrate binding protein might have been involved in metabolism and degradation of the pesticide.

1.6 Factor Affecting Phycoremediation of Pesticides

Microalgae have the capacity to degrade a wide range of pesticides owing to their robust metabolic machinery. However, several factors influence pesticide degradation by microalgae. Some of the key factors are discussed below.

1.6.1 Biological Factor

Phycoremediation of pollutants such as pesticides by a selected microalgal strain depends on its physiology, survival and growth behaviors, species density, tolerance, and previous exposure to the specific pollutant. Moreover, a good synergy and compatibility of the organism with the existing microbiota play a key role is phycoremediation [66–68]. According to previous reports, a consortium of algae and bacteria performs better as a bioremediating candidate than individual algal or bacterial strain [67, 69, 70].

1.6.2 Chemical Factor

The characteristic features of the xenobiotic compounds such as physical and chemical properties (properties, i.e., hydrophobicity, solubility, and volatility) and concentration play a key role in phycoremediation [70–72].

For instance, light aromatic and saturated compounds are more easily degraded than polar and high molecular weight compounds [73].

1.6.3 Environment Factor

Environmental factors such as temperature, pH, light duration and intensity, and oxidation-reduction potential, salinity, and dissolved oxygen of the medium are key players in the process of phycoremediation of pollutants such as pesticides. These factors may limit the growth and survivability of the microalgae and may influence the media geochemistry and consequently affecting the efficacy of the process [71, 70, 74].

1.7 Benefit and Shortcomings of Phycoremediation

The major benefits [49] and shortcomings of phycoremediation are discussed below.

1.7.1 Benefits

1. Phycoremediation technology is a cost-effective technology. There is no requirement of sophisticated instruments and expensive chemicals. Microalgae can efficiently remediate environmental contamination without any extra cost.
2. The biomass generated during the process of remediation can act as a potential feedstock for the production of various products such as bio-chemicals (e.g., pharmaceuticals), bio-fertilizer, and bio-fuel.
3. Microalgae are photosynthetic creatures; thus, they consume the CO_2 generated during the phycoremediation process and help in maintaining CO_2 balance.
4. Conventional remedial methods generate a large amount of sludge which may be hazardous for the environment. But the sludge generated after phycoremediation contains algal biomass which can be used for energy generation and production of other value-added products.

1.7.2 Shortcomings

1. Bioremediation has several shortcomings. For instance, bioremediation depends a lot on the nature of the organism.

Biodegradation of xenobiotics such as pesticide is not a benign response of the microorganism; on the contrary, it is a survival strategy. Most microorganisms carry out biodegradation under conditions which fulfils its necessities. Thus, certain modification of environment might be required to enable the organism to degrade pollutant in an efficient manner [75].

2. Low compatibility of the microalgal strain with the existing microflora and fauna can significantly affect the phycoremediation process.
3. Environmental factors such as pH, temperature, and salinity may influence the feasibility and success of the phycoremediation process.
4. Phycoremediation of pesticide is a slow process which makes its practical feasibility questionable.

1.8 Conclusion and Future Prospects

Bioremediation has proved to be an excellent tool for environmental remediation of pesticides originating from agricultural activities. There are a number of conventional techniques which are employed for pesticide remediation. But the cost associated with these methods is huge which made humans look for alternative remediation methods such as bioremediation. Traditionally, bacteria and fungi have been exploited for bioremediation but recently scientists and researchers have given sufficient attention to microalgae as a bioremediation candidate pertaining to its low nutritional requirements and versatile metabolic activity. Further, microalgae-based remediation may be integrated with other technology such as biofuel production, making them superior to its fungal and bacterial counterparts. However, there is an urgent need of more advance studies using proteomics and genomic tools to identify key genes involved in pesticide degradation. These genes can be used for development of transgenic microalgae for an efficient bioremediation of pesticides.

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