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Review

Microalgae-Enabled Wastewater Treatment: A Sustainable Strategy for Bioremediation of Pesticides

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Abstract: Pesticides have been identified as major contaminants of various waterways. Being classified as potential endocrine disrupting compounds, pesticides in aqueous system are highly hazardous to aquatic organisms and the ecosystem. The treatment of pesticide-containing wastewater can be performed through several means, but a wastewater treatment strategy which emphasizes both treatment efficiency and sustainability is a necessity of current time. In this context, bioremediation has been increasingly promoted as an alternative technique for the remediation of diverse pollutants. Particularly, bioremediation which involves the utilization of microalgae for the removal or conversion of pesticides to the harmless or less harmful compounds is becoming a trend. Exploiting microalgae as a tool for wastewater treatment presents multiple advantages over conventional treatment technologies, which include an opportunity to simultaneously treat pesticide-containing wastewater and nutrient recovery for microalgae cultivation as well as less formation of toxic sludge. This review discusses the roles of microalgae in mitigating pesticide pollution issue, while offering an opportunity for nutrient recovery from various wastewater sources. Based on the current laboratory studies, the use of microalgae bioremediation as a promising strategy for pesticide treatment has been rationalized. The establishment of more pilot scale studies is highly encouraged to further facilitate the implementation of this treatment approach for practical application.

Keywords: microalgae; pesticide; bioremediation; wastewater treatment

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1. Introduction

Pesticides, naturally occurring or chemically synthesized, are a broad array of chemical substances used for pest control through their disruptions on the physiological activities of the targeted pest. For centuries, the use of pesticides has been instrumental to ensure agricultural productivity [1]. When used properly, pesticides can protect humans and animals from many vector-mediated diseases and suppress infestation outbreaks [2]. Despite the importance of pesticides in helping to achieve better food quality and quantity to match with the global demands, their worldwide usage has raised concerns due to the fact that pesticides can impose negative impacts on the unspecified target organisms [3]. It has been stated that about 95% of the pesticides applied did not reach the target pest but were deposited in their surrounding environments [4]. In recent years, pesticides have been regarded as emerging contaminants which can impose serious impacts on human health and aquatic ecosystems, even at low concentration [5]. The diversity of native species, both the target and nontarget ones, can be altered with the introduction of pesticides such as herbicides and insecticides, eventually leading to the disruption of ecological balance and habitat loss. Some classes of pesticides are known to bioaccumulate in organisms and biomagnify at higher trophic levels [6].

Although some countries have banned the residential uses of poisonous pesticides, the agricultural applications of pesticide are still widely permitted [7,8]. While some pesticides can be considered harmless to the users and the current risk assessment also shows insufficient evidence on the lethal and acute effects of pesticides, chronic effects such as birth defects, tumor productions and neurotoxicity have been associated with the long-term exposure to the active ingredients of certain types of pesticides and their metabolites [9,10]. Pesticides can be degraded in the natural environment through the metabolic activities of microorganisms or through chemical decomposition [11]. Nevertheless, many recalcitrant pesticides are stable over time and remain readily detectable in the environment, implying that degradation by soil bacteria lacks efficiency [12]. The high stability and water solubility of the residues of some pesticides make them persistent in the water ecosystem. The degradable products of pesticide, i.e., the metabolites, can also result in water contamination [13]. Lately, drinking water has been identified as a possible route for human exposure to pesticides. The pesticides in drinking water supplies, even at low-level contamination, are perceived as a potential source of health problems due to their functions as endocrine disruptors [14]. The presence of pesticide residues in these water systems has become a continuous concern as it presents a potential threat to public health and water security.

Various strategies have been implemented to alleviate the detrimental impacts of pesticide contamination in aquatic system. Devising potent detection and establishing treatment methods for pesticide contaminated water have been actively pursued. Pesticide remediation can be accomplished through physical, chemical and biological approaches [15]. These processes depend largely on multiple factors such as the type of matrix, nature of pesticide, water chemistry and cost of investment, among others. Therefore, the selection of pesticide treatment technology should take the advantages and limitations of each of them into account. With current emphases on sustainable wastewater treatment, bioremediation which involves the elimination of contaminants through the catabolic ability of microorganisms becomes an important topic. The growth of specific microorganisms is stimulated by utilizing the pesticide contaminants as sources of food and energy. Through bioremediation, contaminants can be converted into less complex structures and harmless gases such as carbon dioxide and water as by-products. Bioremediation by microalgae, also known as phytoremediation, is now a highly preferred process for wastewater treatment [16].

Microalgae are fast growing aquatic organisms that exist naturally in various habitats and throughout the entire oceanic ecosystem, including in freshwater and saline water ecosystems. Microalgae are at the basal level of food pyramid and act as the initial point of trophic transfer; hence, they are crucial in maintaining the equilibrium of aquatic ecosystem. The role of microalgae in recovering important nutrients such as phosphorus and nitrogen from secondary effluents has been increasingly investigated in the past few years. This approach not only reduces the occurrence of eutrophication and long-term pollution problems caused on some persistent organic micropollutants [17–19], but microalgae cultivation in wastewater also offers tertiary treatment coupled with the production of commercially attractive biomass which can serve many purposes in industries [20]. In addition, the biomass produced from microalgae cultivation can be used as feedstocks of a diverse range of products that hold numerous applications in the industries and for bioenergy generation at commercial scale. Microalgae have been used to treat micropollutants such as pesticides, dyes, heavy metals and drugs originated from various industrial sectors, including domestic effluents, agricultural runoffs and pharmaceuticals [21–27]. Microalgae have demonstrated the ability to degrade and detoxify a wide spectrum of organic and inorganic pollutant through bioadsorption, bioaccumulation or biodegradation [28,29]. These pathways provide attractive means to remediate pesticides found in various point of entries. The metabolic mechanisms responsible for pesticide removal by microorganisms have been summarized by Nie et al. [30].

The feasibility of microalgae-enabled bioremediation for a wide range of emerging contaminants including pesticides has been comprehensively reviewed and discussed [31–35]. It has been generally observed that the overall treatment efficiency can be boosted by harnessing the unique capability of microalgae to simultaneously achieve pollutant removal and nutrient recovery. Tremendous efforts have also been made in the studies related to the bioremediation of pesticides. Much research has been focused on studying the efficiency of newly isolated strains in remediating pesticides via different mechanisms. Sheng et al. provided insights into the bioremediation of pesticides using bacteria, fungi and microalgae, in which the mechanisms involved in the contemporary biodegradation approaches, especially those based on bacteria-microalgae consortium, were highlighted [36]. The bibliometric analyses performed by Verasoundarapandian et al. evidenced a significant increase in the research trend on bioremediation of pesticides based on microalgae in the past 5 years [37]. Hena et al. reviewed the potential and effectiveness of microalgae in removal pharmaceuticals and personal care products. The microalgae species and their ability to acclimatize are important factors affecting the pollutant removal efficiencies [38]. The metabolism and pathways of microalgae used for the remediation of emerging pollutants in wastewater have been reported by Maia et al. [39]. With more pesticide compounds being identified as potential candidates to become priority pollutants in near future, there is a need to substantially improve the current wastewater system and to look at the potential alternatives. Although extensive investigations have been made in pesticide remediation using microalgae, these studies have not been systematically reviewed. The present review aims to provide an overview and analyses on the potentials of microalgae-enabled wastewater treatment for pesticide bioremediation. By comparing the features of currently available pesticide treatment technologies, the advantages of microalgae are highlighted. Based on the specific removal mechanisms, the roles and efficiencies of microalgae in removing pesticides from wastewater are evaluated. As assimilation of nutrients by microalgae during the treatment process is an attractive feature for microalgae-enabled wastewater treatment, this review also pays attention to the capability of the treatment process to simultaneously realize pesticide removal and nutrient recovery. The major stumbling blocks in materializing microalgae bioremediation and the current knowledge gaps are identified. The corresponding future directions are highlighted to facilitate the implementation of this interesting approach for practical applications.

2. Pesticides Classification and Characteristics

Pesticides can be categorized in many ways, namely origins, targeted species, chemical characteristics, active ingredients, mode of action and toxicity, just to name a few [40,41]. Figure 1 shows the general classifications of pesticides based on their target, origins, chemical composition, physico-chemical properties and toxicity. Pesticides can be man-made or occur naturally. Many synthetic pesticides have been produced using different chemicals that vary in their compositions, depending on intended application of the pesticides. Herbicides have wide usage in agricultural and wildland ecosystems to control the growth of undesirable weed populations, hence increasing the productivity of crops to achieve an economically profitable level [42]. Therefore, in parallel to the growth of agricultural activities, the usage of herbicide is the fastest growing section, which covers almost 50% of the pesticide industry [43]. Glyphosate and triazine compounds such as atrazine are herbicides that have been heavily used worldwide. These compounds can easily translocate in the ecosystem so their residues can be detected in soil, water, and biota. As the name implies, insecticides are chemicals used to kill insects to protect cultivated plants or to mitigate disease-carrying insects in some regions. Insecticides harm insects by disrupting their nervous system [44]. Neonicotinoids such as acetamiprid, thiacloprid and imidacloprid are a class of synthetic neuro-active insecticides widely used for insect control. Neonicotinoids impose selective toxicity through pharmacophores that can bind with the receptors of insect [45]. The toxicity effects of neonicotinoids on different species of invertebrates such as bees as well as on the metabolisms of aquatic organisms have been reported [46]. Other major categories of pesticide are rodenticides used to bait and control rodent population, as well as fungicide and bactericides which acts as an inhibitor of fungal spore germination and bacteria growth, respectively. Some pesticides can be used to control more than one class of pests. Based on the WHO classification, pesticides can be divided into several classes, varying from Type III for slightly hazardous pesticides such as malathion to Type Ia for extremely hazardous such as parathion.

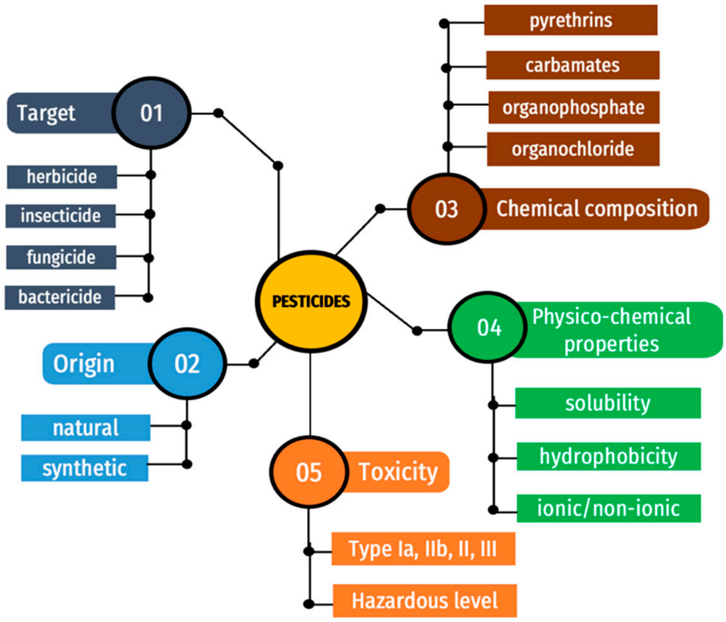


Figure 1. General classifications of pesticides.

The more useful and common way of classifying pesticides is based on their chemical composition and active ingredients as this classification provides a direct indication of the physico-chemical properties of the pesticides, hence determining the mode of application, efficiency, precautionary steps during their application and their fate in the environment. The chemical compositions of insecticides can be divided into four primary groups, i.e., organochlorines, organophosphorus, carbamates and pyrethrin and pyrethroids [47]. Organochlorides are chlorinated hydrocarbon derivatives commonly found in insecticides. Common organochloride pesticides include dichlorodiphenyltrichloroethane (DDT), lindane, and endosulfan [48]. Organochlorine pesticides are known for their high persistence in the environment [49]. This property is closely related to presence of a halogen electron withdrawing group that results in an electron-deficient condition such that the compounds can withstand aerobic degradation. The human exposure to organochloride may cause neurological damage and endocrine disorders. Organophosphates such as malathion, parathion, diazinon, fenthion are ester of phosphoric acids [50]. They are composed of organic moieties and central phosphate groups such as thio- or dithio-phosphate. The wide variety of substituents used for the synthesis of organophosphate compounds causes great variations in their physicochemical properties such as polarity and resistance to degradation. Organophosphates are less stable and degrade faster than organochlorides by hydrolysis [51]. Organophosphate affects the enzyme functions of insects and some mammals through irreversible covalent inhibition. The more severe acute toxicity of organophosphate causes the elevated risk associated with the use of this class of pesticides. Prolonged exposure of organophosphate results in increased risks for central nervous, cardiovascular and respiratory diseases. Carbamate compounds used in insecticides are esters derived from carbamic acid, whereas those used in herbicides are synthesized from the derivatives of carbamic acid such as thiocarbamic acid and dithiocarbamic acid [52]. Sharing the same toxicity mechanisms as organophosphate, carbamate pesticides are harmful to nervous system, but normally with reversible and less severe effects. Triazine compounds which contain triazine isomers as part of their core structures are a major class of herbicides [53]. The commonly used triazines are symmetrical triazines such as chloro-s-triazines thiomethyl-s-triazines and methoxy-s-triazine. They act as inhibitors of photosynthesis by blocking the movement of electrons through the binding with the protein in the transport chain.

The studies of pesticides which include their detection, distribution in soil and water bodies, modes of action, transport mechanisms and pathways have been performed extensively [54–56]. Identifying the sources of pesticide residues is the key to mitigate pesticide pollution in soil and water. Commonly, besides depositing on fruits and vegetables, pesticides sprayed on crops find their way into the aquatic systems including ground water and surface water through irrigation and runoff [57]. The excessive leakage of pesticides into the environment is also related to the poorly regulated disposal guideline and the mishandling of users [58]. The cleaning of pesticide containers and spraying machines, as well as the improper disposal of pesticides, also leads to the enrichment of pesticides in the receiving water bodies. Pesticide production wastewater is the wastewater generated from the pesticide manufacturing industries [59]. Treatment of pesticide production wastewater is required prior to their mixing with domestic wastewater. The migration of pesticides through soil into water bodies depends on many factors including the soil texture, pesticide concentration, pesticide–soil interaction and stability of pesticides. Other important physiochemical properties such as the water solubility, vapor pressure, and octanol–water partition coefficient (K_{ow}) of pesticide compounds also dictate their modes of transportations and possible sinks in the environment. Although the actions of pesticides are meant to be specific to the target species, they may also exhibit toxic effects to other nontarget species upon the exposure to the aquatic creatures. Their toxicity towards freshwater fish and estuarine have been widely reported [60,61]. The subsequent exposure of humans and animals to these pesticide residues occurs via ingestion of foods containing them, or absorption through skin or lungs.

3. Treatment of Pesticide-Containing Wastewater

The selection of the pesticide treatment method majorly depends on the compositions of pesticides in wastewater, treatment cost and simplicity of the operation. Therefore, a comprehensive analysis of influent characteristics and the coupling of the most suitable treatment technology are required for the design of treatment facilities targeted for removal of emerging pollutants such as pesticides in wastewater. It is also important to correlate the characteristics of pesticide compounds with the environmental conditions as the characteristics such as solubility, reactivity and surface charge can be considerably changed by the surrounding matrices. Physical, chemical and biological methods have been widely used for the removal of pesticides in aqueous medium [62–64]. Constructed wetland treatment based on pseudo-natural engineered conditions has been commonly used to mitigate pesticide in agricultural run-off [65]. Various microbial, biological, physical, and chemical mechanisms can take place to facilitate the elimination of pesticides in a constructed wetland treatment system [66]. The removal of organophosphate has been achieved with removal efficiency of >80% [67]. However, the removal efficiency of pesticides in constructed wetland varies by the pesticide types [68]. Adsorption and filtration are physical processes widely used in wastewater treatment plants. They are suitable for a wide range of wastewater treatment, mainly due to their simplicity, high removal capacity and cost-effectiveness [69–73]. Using functional nanosized adsorbent such as multiwalled carbon nanotubes, removal efficiency of up to 90% has been reported for diuron [74]. Lately, adsorbents derived from agricultural waste demonstrate high potential in treating pesticides in a sustainable way [75]. The main feature of physical treatment processes is their ability to capture or trap pesticide contaminants from the contaminated water, so that the separation between pesticide compounds and the remaining compositions can be accomplished. Although most physical treatment processes can be feasibly established at large scale and popular with many industries, the formation of hazardous sludge and its subsequent disposal operation and cost often a major concern of these processes.

On the other hand, chemical and biological processes involve the chemical alteration of the compositions and structures of the targeted pesticides, hence converting them into other forms of compounds that are normally simpler and less toxic. Degradation of pesticides through advanced oxidative processes (AOPs) has been widely reported [76–79]. Photocatalysis, fenton oxidation and ozonation have demonstrated high feasibility for pesticide removal. AOPs are attractive for the oxidative degradation of organic pollutants on account of their high efficiency in short reaction time and less sludge production compared to physical methods [80]. The major bottleneck of chemical treatment process is large doses of several different chemicals that are typically required to enable the oxidation processes. In addition to cost concern, the use and disposal of some harsh chemicals also create an environmental issue. Biological method involves bioremediation using plants such as algae or microorganisms such as yeast and bacteria to absorb, accumulate or degrade the pesticide pollutants [81]. Compared to physical or chemical methods, biological methods can be performed at lower cost and in more environmentally friendly manners [82,83]. Activated sludge is the most common biological method used in current wastewater treatment system to remove organic pollutants [84,85]. Microorganism strains such as *Pseudomonas* and *Bacillus* have been isolated from various sources including pesticide-contaminated soil for the biodegradation of pesticide [86,87]. An advantageous feature of microalgal biodegradation is that it transforms the harmful pesticide compounds to less toxic molecules rather than just serving as a biofilter that separates the pesticide compounds from their matrices. Recently, mixed bacteria–microalgae consortia have been increasingly used for the bioremediation of pesticides [88]. Studies have evidenced that bacteria secrete chemicals that can enhance the interaction with microalgae. The co-existence of denitrifying bacteria and microalgae can also improve nutrient removal efficiency [89].

Hybrid treatment technologies offer opportunities to tackle the limitations of single treatment process and to amplify the treatment efficiencies by combining the unique advantages of each process [90]. In addition to their capability in delivering high pesticide removal efficiency, technologies such as oxidation and filtration processes also show great promise in terms of their versatility and compatibility to integrate with each other to further improve the efficiency of treatment processes [91]. It is worth noting that despite the efficiencies of these technologies in removing pesticide in lab settings, many full-scale wastewater treatment systems are incapable of removing most of the pesticides below the required limit as they are not designed for this purpose, but to improve the water quality by removing major contaminants. The establishment of specialized units to treat a particular type of wastewater on site will ensure the use of more appropriate techniques based on the water composition.

4. Bioremediation of Pesticides Using Microalgae: Why and How

Microalgae are phytoplanktons which feature biochemical properties such as oxygen-mediated photosynthesis. Microalgae can be found in various species, and most of them can grow and survive under hostile conditions such as high salinity and extreme temperature. Compared to other plants, microalgae can produce higher biomass yield without the use of non-arable land. Therefore, microalgae represent a sustainable resource for biomass production to answer growing demand for bioprocessing and production of environmentally friendly products. Microalgae can tolerate and grow well in saline wastewater with high load of organic matter [92]. The acclimation of microalgae allows them to tolerate the toxicity of pharmaceutical, pesticide and mining wastes [93–95]. By utilizing the inherent biological mechanism of microalgae, the treatment process can be accomplished without producing undesirable by-products that may result in secondary pollutions. Furthermore, the cultivation of microalgae can mitigate carbon dioxide and absorb the micronutrients in the effluents during their growth process [96]. Microalgae can alter and stabilize the physico-chemical–biological characteristics of wastewater. For instance, they can reduce the chemical and biochemical oxygen demand and retard the growth of selected bacteria species [97,98]. In addition, microalgae are capable of degrading phenolic and dye pigments that are commonly found in wastewater, thus decreasing the color intensity and improving the availability of sunlight in the water bodies [99]. The decolorization of wastewater can also help in easing the treatment process at wastewater treatment plants. Interestingly, the cultivation of microalgae in wastewater allows simultaneous pollutant removal and nutrient recovery from a wide range of wastewater. The recovery of nutrients from wastewater using microalgae has been widely reported. Nitrogen compounds in the form of nitrites, nitrates and ammonium are assimilated via various pathways to sustain their growth, meanwhile phosphorus in the form of phosphate is used to facilitate the metabolic activities [100–102]. Microalgae could achieve removal efficiencies of up to >95% for nitrogen and phosphorus [103–105]. One of the most extensively studied species of microalgae for wastewater treatment is *Chlorella, Chlamydomonas* and *Scenedesmus* sp. [32] due to their strong adaptability in stressful and harsh environmental conditions. Among all, *Chlorella vulgaris* has attracted particularly wide attention owing to its advantages in terms of ability to grow in autotrophic, heterotrophic, or mixotrophic conditions which confers them the advantage to consume different types of soluble substrates in the aquatic system. The consortia of microalgae and bacteria have also been increasingly explored in this area, as it represents a significant advance in improving the process effectiveness [106].

The phytoremediation of pesticides through microalgae can take place through one of the three main pathways as shown in Figure 2, namely biosorption, bio-uptake or bioaccumulation and biodegradation. Biosorption by microalgae is a passive and metabolic-independent process. The process occurs when the pesticide molecules are adsorbed to the cell wall of the microalgal cells. The fibril carbohydrate matrix, sulfated polysaccharides and the intercellular spaces of the cell wall could facilitate the adsorption of organic contaminant in wastewater [107]. Microalgae possess high surface area to biovolume ratio, hence can serve as a good medium for sorption and the subsequent interaction with pesticides. In addition, the surface of microalgae offers diverse multifunctional groups with uniform distribution of binding sites to facilitate the biosorption so that various mechanisms such as complexation, ion exchange and precipitation can take place through electrostatic interaction at micro level [108]. The biosorption of transition metals by *Chlorella vulgaris* was accomplished through functional groups such as sulfate, carboxyl, and hydroxyl groups [109] while the biosorption of 2,4-dichlorophenoxyacetic (2,4-D) herbicide was predominantly established through their interaction with hydroxyl, carboxyl, and amine active surface groups of *Gracilaria verrucosa* [110]. The mechanisms and roles of functional groups during biosorption are highly dependent on the characteristics of pollutants such as their charges and polarity. Biosorption is a major mechanism involved in the removal of heavy metal ions by microalgae from wastewater [111]. Apart from the functionality of the microalgal cell wall, the extracellular polymer substances (EPS) secreted also affect the biosorption efficiency. Like typical adsorption process, microalgal biosorption process is also influenced by many factors including pH, temperature and contact time. The interactions occur between different contaminants and microalgal biomass can also affect the strength of the sorption, therefore the relevant parameters such as ionic strength should not be ignored [112].

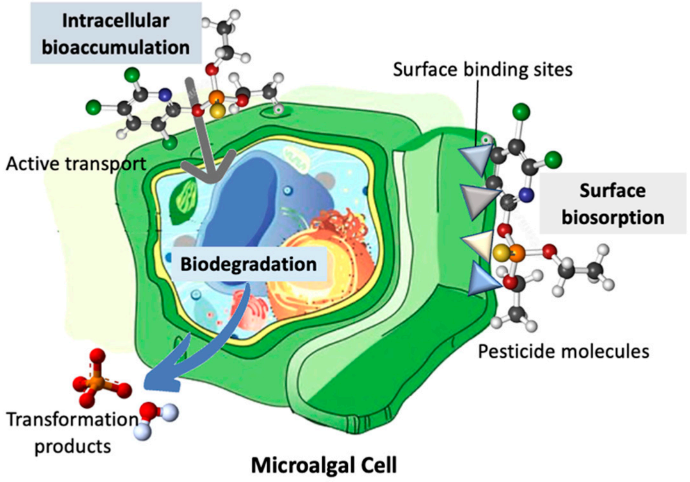


Figure 2. Schematic illustration of phytoremediation of pesticides by microalgae through biosorption, bioaccumulation and biodegradation.

Bioaccumulation, also termed active biosorption, is a process where the pesticide molecules are first attached to the surface, followed by their migration into the living microalgal cell through active transport to bind with intracellular proteins and other organelles [113]. Under the same condition, bioaccumulation takes place at lower rate that the biosorption. The efficacy of bioaccumulation is governed by the bioconcentration factor which is defined as the ratio of concentration of a pollutant accumulated in the microalgae to the concentration of exposed environment at equilibrium. The bioaccumulation of toxic pesticide compounds in the microalgal cells prompts the production of reactive oxygen species (ROS) from cell organelles [114]. The excessive ROS causes oxidation of DNA and membrane lipids, leading to disruption to cell components and functionalities. The side effects of bioaccumulations to the microalgal cells are countered by the production of antioxidative enzymes that can eliminate excessive ROS by scavenging these free radicals [115]. It was reported that insecticides acephate and imidacloprid with concentration of 15 mg/L induced an adaptive biochemical change in freshwater microalgae *Chlamydomonas mexicana*, where superoxide dismutase activity (SOD) antioxidant enzyme was significantly increased as part of the defense mechanisms [116]. The photosynthetic algae native to polar regions, *Cocomyxa subellipsoidea* hydrolyze and breakdown paraoxon, malathion and diazinon organophosphates through the formation of ROS have been demonstrated [117]. Interestingly, the ROS-dependent mechanism exhibited little to no toxic effects on the algae.

Biodegradation is the most important and effective mechanism for contaminant removal through microorganisms. It involves catalytic metabolic degradation which converts complex pesticide compounds into simpler molecules or completely mineralizes them [118]. Microalgae can decompose organophosphorus compounds and use the compounds of nitrogen, phosphorus and carbon as a source of nutrients [119]. The biodegradation of pesticides by microalgae relies on the metabolism of various enzymes that play different roles during the biodegradation process. The biodegradation of pesticides involves multiple steps which can be generalized as (i) the activation of pesticides through oxidation, reduction, and hydroxylation to increase their hydrophilicity, solubility, and degradability, (ii) the transfer to enzyme to pesticides to form conjugates and (iii) transportation of conjugates into vacuoles [30]. For pesticides with high water solubility, their higher occurrence and mobility in aqueous medium allows higher bioavailability for biodegradation, specially under long hydraulic retention time [120]. Pesticides can be simultaneously bioaccumulated and biodegraded by microalgae. Fresh water Scenedesmus obliquus demonstrated simultaneous bioaccumulation and biodegradation of triadimefon to its metabolite, triadimenol. The mixed mechanisms allowed a more rapid and efficient removal of pesticides. It was also observed that the biodegradation of triadimefon was stereoselective with degradation of S-

(+)-enantiomer was more favourable, indicating that chirality of pesticides is an important parameter to be considered for microalgal biodegradation [121].

The treatments of pesticides using microalgae can be accomplished in several ways, as shown in Figure 3 [122]. The most conventional way of cultivating microalgae is through an open microalgal system such as high-rate algal ponds (HRAPs). HRAPs are designed as shallow raceways that can co-culture microalgae and bacteria to allow the degradation of organic matter by heterotrophic bacteria while consuming oxygen produced from microalgal photosynthesis [123]. Currently, huge efforts have been dedicated to the establishment of photobioreactor (PBR), a closed system design for the cultivation of microalgae using solar or artificial light sources to enable photosynthesis of the photoautotrophic organisms [124]. Compared to its membrane bioreactor (MBR) counterpart, PBR does not require electromechanical aeration for the liquor mixing due to the photosynthetic activity of microalgae, hence it is more advantageous in terms of energetic cost involved for the wastewater treatment. Although the cost of operation and maintenance and the energy consumption of PBR are higher compared to that of closed systems, PBR allows better control of environmental parameters including temperature and light irradiation for higher biomass productions. As the microalgae culture has better protection, the risk of contamination is also much lower in a closed system [125].

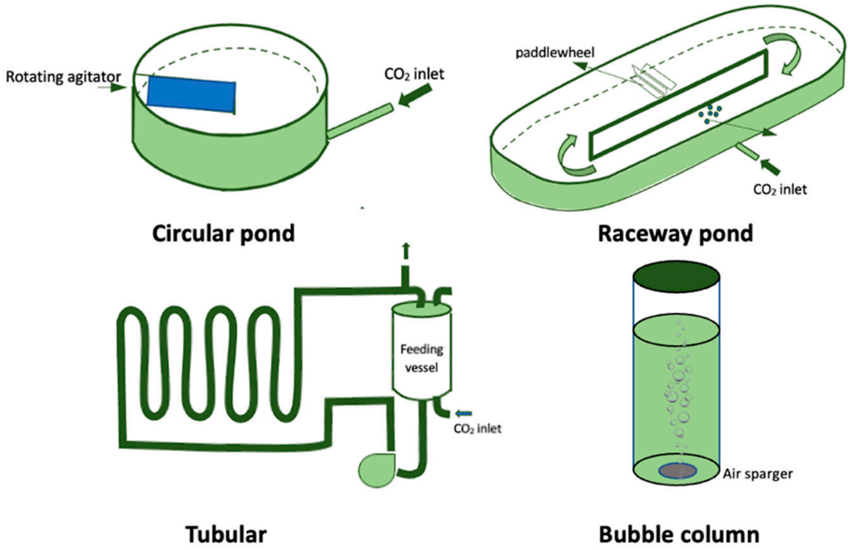


Figure 3. Different configurations of microalgae photoreactor system.

5. Recent Research Progresses in Pesticide Bioremediation Using Microalgae

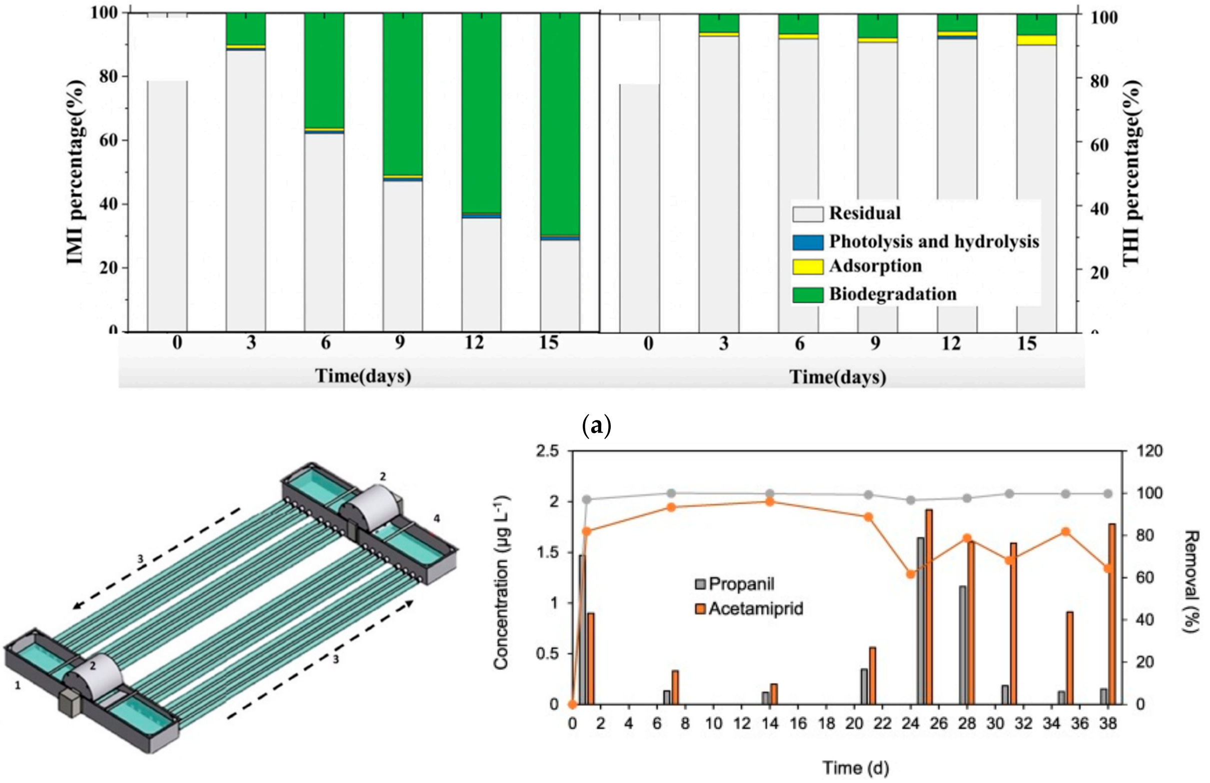
5.1. Pond-Based Treatment

The removal efficiency of 26 common wastewater organic micropollutants including 2,4-D diazinon and atrazine pesticides has been evaluated based on pilot-scale HRAPs [126]. A significant seasonal variability for the micropollutant removal was observed due to the change in the predominant algae species and the variations in their metabolic activities during warm and cold seasons. The removal of NH_4–N decreased from 99% during summer to 90% during winter in a HRAP operating at a hydraulic retention time of 4 days, indicating that the environmental conditions also affected the nutrient removal efficiency. Although the removal efficiency of HRAPs was comparable to that of conventional wastewater treatment plant, it was observed that the removal of pesticides (<76%) was generally lower than that of other pharmaceutically active micropollutants (>95%). This was explained by the variations of the complex chemical compounds which resulted in the occurrence of different removal processes that took place simultaneously in the microalgal wastewater treatment system.

5.2. Flask-Based Treatment

Recent laboratory demonstrations have witnessed the efficiency of microalgae in treating different types of pesticides present in agricultural run-off. Castellanos-Estupiñan reported >75% removal of chlorpyrifos from rice plantation runoff using *Chlorella* and *Scenedesmus* sp. [127]. While both nitrate and phosphate can be significantly reduced by >80%, the removal efficiency of *Scenedesmus* sp. was significantly higher than that of *Chlorella* sp. The removal efficiencies of pesticides from agricultural run-off using microalgae systems operated under batch feeding operational mode were evaluated based on 11 types of pesticides [128]. Due to the differences in their chemical structure and resistance towards biodegradation, the pesticide removal efficiencies varied significantly: poor removal efficiency of <10% has been observed for mecoprop, atrazine and simazine, while high removal efficiency of 91% and 99% has been achieved for endosulfan and malathion, respectively. For pentachlorobenzene which can be easily photodegraded, the presence of microalgae has hampered the photo-oxidation upon its adsorption to the microalgae surface, resulting in poor removal from the agriculture run-off. Under the same operating conditions, it was observed that continuous operation mode can further improve pesticide removal efficiencies as the higher microalgae turnover resulted from the exponential growing phase in the continuous feeding operation facilitated more adsorption and degradation of pesticides. In the case of batch mode operation, the growth of microalgae reached a stationary phase and the capacity to remove pesticides declined at this point.

A microalgae–bacteria consortium was formed using *Scenedesmus* sp. strains isolated from a wastewater treatment plant to simultaneously remove imidacloprid, thiacloprid and nutrient from the wastewater [129]. The consortium achieved removal of 71.24% for imidacloprid and 9.71% for thiacloprid, which was 55.54% and 2.71% higher than that of without microalgae inoculation. A removal rate of total nitrogen and phosphate was also higher. As depicted in Figure 4a, biodegradation was identified as the primary mechanism responsible for the removal of imidacloprid and thiacloprid. However, due to the difference in their chemical structures, the removal efficiency of imidacloprid with N-nitroimine was higher than that of thiacloprid with N-cyanoimine as the *Scenedesmus* sp. TXH had better transformation effects on imidazole, the most reactive site of IMI that is susceptible to various chemical reactions such as oxidation, reduction, and hydroxylation. The environmental factors such as light intensity and temperature have been identified as key parameters to be optimized during the treatment as they can interfere with the interaction of microalgae and bacteria in the symbiotic system. Temperature influences the stability and adsorption ability of the microalgae–bacteria consortium while light plays an important role in the enzyme activity of microorganisms [130].



(**a**)

(**b**)

(**c**)

Figure 4. (**a**) The mechanisms involved in the removal of imidacloprid and thiacloprid [129]. (**b**) Schematic diagram of pilot scale semi-closed PBR used for the treatment of agricultural run-off [131]. (**c**) Concentration and removal efficiency of acetamiprid and propanil in the pilot PBR effluent as a function of operation duration [132]. (Reprinted with permission.)

5.3. PBR-Based Treatment

García-Galán evaluated the efficiency of a pilot scale semi-closed PBR in removing pesticides in peri-urban agricultural areas [131]. As illustrated in Figure 4b, the PBM system consisted of two open tanks connected using horizontal tubes, operating with useful volume of 11.7 m^3 and hydraulic retention time of 5 days. Similar to many laboratory attempts, the PBR treatment demonstrated inconsistent removal efficiencies depending on the pesticide compounds. Among the 16 types of medium to highly polar pesticides detected in the run-off, 10 types of pesticides including alachlor, linuron and cybutrine have been fully eliminated. Although removal of up to 88% has been achieved, 2.4-D, MCPA, diuron, terbutryn, diazinon and imidacloprid were identified as more resilient pesticides in the PBR system. Particularly, the conversion of some metabolites into the original pesticide compounds during the PBR treatment has resulted in higher concentrations of diuron, terbutryn, diazinon in the treated effluent.

The removal of acetamiprid and propanil using an outdoor pilot-scale tubular PBR was evaluated in a continuous operation for 38 days [132]. As shown in Figure 4c, The removal efficiency of propanil increased over time due to biomass acclimation and eventually >99% of the pesticide was removed through biodegration in the PBR with an HRT of 8 days under semi-continuous mode. Complete mineralization of its intermediate by-products was also observed. On the other hand, acetamiprid required a longer time for its complete degradation in the PBR, with meant removal efficiency of 71% during the steady state. While acetamiprid has lower biodegradability, biomass washout in the pilot PBR has further resulted in inefficient biodegradation by the declining biomass concentration. The finding implies that biomass washout in PBR should be minimized so that the biomass can be sufficiently used to sustain the biodegradation, especially for pesticides with lower biodegradability. One way of addressing this issue is by introducing membrane modules in the PBR. Through the integration, not only the washing effect can be minimized, but the decoupling of solid retention time from the hydraulic retention time can also be materialized through the confinement of microalgal biomass.

5.4. Integrated Microalgae System

Microalgae are mostly suspended in the typical microalgal reactors. The immobilization of microalgae during the wastewater treatment involves the entrapment of the microalgal cells within a matrix to limit their mobility while maintaining their metabolical activity as long as possible [133]. The immobilization of microalgae in a host matrix has been accomplished for several reasons [134]. The immobilization technique can increase the cell survival and metabolic activity as well as ease in the separation of microalgal biomass after treatment. In addition, the immobilization of bacteria–microalgae consortium can improve the symbiotic interactions between the two species [135]. Many host materials such as alginate and luffa sponge have been reported for microalgae immobilization [136]. The stability of host and the immobilized microalgae is an important aspect especially for scaled up long-term application. Rerrando and Matamoros reported the immobilization of microalgae–bacteria consortium in polyurethane foam for the treatment of groundwater containing micropollutants [137,138]. They observed an enhanced bacteria biodegradation processes in the symbiotic consortium for the biodegradation of pesticides (bromacil, atrazine, diuron, bentazone, and mecoprop). Despite the light blocking effect caused by the host matrix, the removal efficiency of the polyurethane-immobilized microalgae was improved by >30% compared to that of the free counterpart. Compared to microalgae immobilized in luffa sponges which disaggregate in the reactor due to the mechanical stirring effect, the foam material was able to retain its integrity throughout the 60-day operation in the continuous-feeding reactors.

Microalgal fuel cell is a relatively new concept that exhibits significant potential for simultaneous carbon fixation and bioenergy generation while delivering enhanced wastewater treatment efficiency. Deng et al. developed a two-chamber microalgae fuel cells comprised of an anaerobic anode chamber and an aerobic cathode chamber separated by a proton exchange membrane [139]. The removal of imidacloprid was accomplished in the cathode chamber of microalgae fuel cells using *Chlorella* sp. while anaerobic sludge and wastewater were inoculated in the anode chamber. Under closed-circuit condition, the removal of imidacloprid was achieved in the range of 57–62% through biodegradation. Several degradation products of imidacloprid were detected, but they were less toxic than the original imidacloprid and had insignificant impact on the growth of microalgae. The cathodic electroactivity has been promoted with the degradation of imidacloprid where voltage of 200 mV was generated for up to 9 days. In another study using a similar system, the highest removal of thiacloprid (32.5%) was achieved at low pesticide concentrate below 20 ppm with stable bioelectricity voltage of 202 mV [140]. The concentration of thiacloprid above 50 ppm induced oxidative stress to the microalgal cell and depressed the biomass growth.

5.5. Discussion

Microalgae-enabled wastewater treatment is postulated as an alternative for pesticides removal from aqueous environment. The recent achievement made in this field has been compared and summarized in Table 1 to better understand the feasibility of this approach. Bioremediation through microalgae has been accomplished through pond and photoreactors system. For lab-scale studies, batch removal of pesticide has been investigated using simple flask-based set up. Based on the studies discussed in this section, regardless of the types of system, it is observed that the removal efficiency of different classes of pesticides is obviously different due to the difference in their functional groups and chemical composition. Furthermore, the performance of a single type of microalgae can be sometimes inconsistent and different species of microalgae exhibited removal efficiencies that vary for each class of pesticides. Systematic screening is therefore needed to select and validate the removal efficiencies of pesticides by a wide diversity of microalgae species. As the chemistry and external condition of the aquatic environment vary from source to source, the selection of the native strains dominating the identified wastewater environment is a crucial step towards achieving high removal efficiency by the microalgae in a practical manner. In most of the current studies, the efficiency of pesticide removal and nutrient recovery by microalgae has been evaluated based on removal efficiency (%) or removal rate. While these parameters serve as a good indication for the performance of microalgae-enabled strategies in remediating pesticides, it is important to ensure that the treated effluent contains pesticide level that meets the standards set by the local authorities. It is exciting to witness the innovation introduced in this area, one of them is the development of microalgal fuel cell that demonstrates potential for bioenergy generation while treating pesticide-containing wastewater. Although the power output is still below satisfactory level, the efforts aligned with this direction will provide more positive outcomes.

Table 1. Summary of pesticide and nutrient removal efficiency through microalgal bioremediation.

System	Wastewater	Microalgae Species	Efficiency	Refs.
HRAP	Urban wastewater	*Stigeoclonium* sp., *Chlorella* sp.	Diazonon, 2,4-D, atrazine: 40–60% N-NH$_4$+: 99%	[126]
Batch mode flask reactor	Agriculture run-off	*Scenedesmus* sp., *Chlorella* sp.	Chlorpyrifos > 75% N-NO$_3$$^-$: 85% P-PO$_4$$^{3-}$: 82%	[127]
Batch mode flask reactor	Agriculture run-off (batch)	*Scenedesmus* sp., *Chlorella* sp.	Endosulfan: 91% Malathion: 99%	[128]
Batch mode flask reactor	Municipal wastewater	*Scenedesmus* sp. TXH	Imidacloprid: 71.2% Thiacloprid: 9.71% Total dissolve N: > 80% Total dissolved P: > 90%	[129]
Batch mode flask reactor	Ground water	*Scenedesmus* quadricauda, *Chlorella vulgaris.*	Nitrate: 44% Bromacil: 94% Atrazine: 83% Diuron: 88% Bentazon: 54%	[137]
PBR	Agriculture run-off	*Chlorella* sp. and *Stigeoclonium* sp.	MCPA: 88% alachlor, linuron, cybutrine: 100% N-NH$_4$+: 93% N-NO$_3$$^-$: 54% P-PO$_4$$^{3-}$: 100%	[131]
PBR	-	*Scenedesmus* sp., *Chlorella* sp.	Propanil: 99% Acetamiprid: 71% N-NO$_3$$^-$: 24% P-PO$_4$$^{3-}$: 94%	[132]
Microalgae fuel cell	-	*Chlorella* sp.	Imidacloprid: 57–62%	[139]
Microalgae fuel cell	-	*Chlorella* sp.	Thiacloprid: 32.5%	[140]

6. Challenges and Future Research Directions

With the current emphasis on circular economy, microalgae-enabled wastewater treatment is deemed to provide a holistic solution to achieve removal effectiveness and resource conversion potential. Nevertheless, apart from the efforts made in delving into the potential of microalgae for the bioremediation of pesticides, unexplored avenues still exist. The unsolved questions and knowledge gaps must be tackled to promote the implementation of this strategy for practical applications.

Bioremediation has been generally known to take place through biosorption, bioaccumulation and biodegradation. However, despite the understanding achieved to date, it is still challenging to gather collective information on the intercorrelated factors that affect metabolic pathways, optimal conditions for degradation. In addition to the three major pesticide removal mechanisms, the abiotic processes such as volatilization and photodegradation of pesticides in the microalgal wastewater medium should not be disregarded. Furthermore, it is well established that only a small amount of the pesticide applied directly acts on the targeted species while the remaining pesticides tend to interact with soil to form metabolites with longer half-life and more complex properties. The action of these metabolites on the biodegradation should be investigated. In-depth investigations are required to examine the quality of treated effluent after the separation of microalgae from the reactors. In addition, the detection of byproducts is also important to avoid secondary pollutions. As the biodegradation not always leads to complete mineralization of pesticide compounds, attention should also be focused on the toxicity assessment of the resulting mixtures. It is also known that the mode of nutrition could interfere with the ability of some microalgae in remediating pesticides. The addition of external carbon source may be required to improve the durability of microalgae in poisonous concentrations of pesticides. Such observations provide a clue on the suitability of microalgae remediation for different sources of pesticide-containing wastewater.

Elaborated tools and advanced technology help to bridge the current knowledge gap in regard to the mechanisms involved in the bioremediation of pesticides. Combining experimental findings and statistical tools in an integrative way is important in the research in this field. Progress in computational studies to demonstrate the necessary mechanisms and pathways in a much precise manner and more predictably could help optimize the pesticide remediation efficiency while realizing sustainable water treatment strategies. A less complicated computational tool such as response surface methodology (RSM) would be helpful in providing more insights into the interactive relationship of the major influential parameters, so that the pesticide and nutrient removal efficiency can be better correlated. The finding also serves as a basis to optimize the system operated at a larger scale. Dynamic modelling of data can help to predict the behaviour and biological activity of microalgae when dealing with wastewater with different characteristics. The use of instrumentation, control and automation (ICA) systems can be used to gather all the relevant information about the process to improve the efficiency and robustness of the system. However, it should also be pointed out that the complexity of modelling variables involved in pesticide-containing wastewater conditions and their effects on the microalgae cultures will be a great challenge to address.

Pesticide bioremediation through microalgae is a complicated process. Two major aspects can be taken into account, the survival and activity of the microalgae strains and the characteristics of the pesticides. The concentration of pesticide has considerable effect on the efficiency of microalgal bioremediation. The concentration of pesticide in the range of $\mu g\ L^{-1}$ generally has no profound effect on microalgae, but at higher concentration, the toxicity of pesticides may lead to inhibition of cell growth [141]. In addition, for a given pesticide concentration, the metabolic responses exhibited by microalgae may vary by species [142]. Therefore, a detailed profiling on the tolerance of microalgae will be useful to create a database that can serve as a guideline for microalgae selection. A very recent study on the sensitivity response of artic microalgae to pesticide interestingly showed that Arctic species *Micromonas polaris* demonstrated higher resistance to atrazine and simazine than its temperate counterpart [143]. The difference in the ecophysiological characteristics of the two microalgal species have contributed to their variations in terms of growth, cell size, activity, ROS contents and protective mechanisms. Such study highlights the importance of establishing an efficient screening through molecular toolkits to identify the potential of different species so that the underlying molecular mechanisms of bioremediation can be revealed and the best-fit microalgae species can be selected.

The large-scale commercial application of microalgae for the bioremediation of pesticides and other emerging micropollutants remains uncertain due to the conceptual gap between laboratory finding and industrial expectations. Numerous tailbacks should be carefully addressed to promote the large-scale implementation of the bioremediation technology. The experimental studies implemented in bench-scale have been conveniently performed using a simple apparatus set up such as shake flasks under controlled sterile conditions. While such setting provides a quick evaluation of the performance, the operating conditions and the corresponding findings are difficult to scale up to pilot-scale plant systems which usually operate in continuous mode and are fed with complex real water effluents. As the main purpose of setting up a laboratory study is to explore the possibility and to validate the performance of a newly developed approach, the experimental procedures are often simplified by fixing a representative experimental concentration. Most of the current pesticide removal studies have been focused on the use of single pesticides in a single experiment. It is obviously not the case in real scenario. For instance, inhibitory effects on the biodegradation of *Scenedesmus obliquus* have been observed when the mixture of several organic pollutants was tested using the microalgae-enabled wastewater treatment system [144]. The studies on the interactions and competitions among the organic micropollutants are still not well-grounded. The limitations reveal the importance of setting up laboratory experiments by considering the effects of co-existed micropollutants in the real wastewater so that the actual removal efficiency can be practically elucidated. The external environmental conditions have considerable effects on the overall efficiencies of microalgae, not only the pesticide removal but also the nutrient recovery rate. The uncertainties in some technical aspects, especially the inconsistency in performance and challenges in adapting under varying weather conditions, may limit the wider practical application of the bioremediation. For outdoor operation, the fluctuations of the removal efficiency due to the environmental conditions such as during winter and summer should be taken into consideration. It is therefore important to establish pilot-scale studies in a long-term set up throughout the year so that the fluctuations in the environmental factors such as intensity of solar irradiation and wastewater characteristics can be considered when evaluating the efficiency of the system.

While the current advancements have improved our understanding of pesticide remediation through microalgae, future research should be structured to address knowledge gaps and make innovations at the implementation level. Lastly, although bioremediation has been generally regarded as a cheaper approach as compared to the physical and chemical processes, the cost effectiveness at large scale implementation has not been widely evaluated. In addition, the performance stability for long-term operation should be meticulously investigated. Comprehensive techniques and complete assessments are required to address the procedural and ecological issues to facilitate the scale up of its application. The knowledge can be harnessed to build a framework for the establishment of solutions for the current pesticide management challenges. The life cycle analyses which look at the viability of microalgae enabled bioremediation of pesticides should be established to ensure the implementation of the technology as a real cost-effective innovative solution for wastewater treatment.

7. Concluding Remarks

Natural sources such as microalgae can serve as an ecologically safe, cheap, and efficient alternative for removing hazardous pesticide pollutants from aqueous environments. Apart from pesticide removal, bioremediation through microalgae is also promising for many other emerging pollutants. The development of microalgae bioremediation approaches for pesticide removal is still at its beginning stage, but some promising results have already been witnessed in some laboratory and pilot scale attempts. Pesticide removal using microalgae still confronted by many technological challenges, but with the attractive features demonstrated by microalgae as a tool for bioremediation, this strategy is deemed as an economical alternative to the existing techniques. With the plethora of information available and the help of innovations made in this field, it is anticipated that this technology can evolve from the laboratory to practical application in near future.

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