Tropical Aquatic and Soil Pollution

*Review*
**Volume 4(1), 2024, 10−26**
https://doi.org/10.53623/tasp.v4i1.403

# Biodegradation of Chlorpyrifos by Microbes: A Review

**Rubiyatno[1]*, Aulia Maulana[2], Jovale Vincent Tongco[3], Arma Yulisa[4], Sang Hyeok Park[4], Md Abu Hanifa Jannat[4], Rega Permana[5,6], Ocean Thakali[7], Michael Lie[8], Aouatif Fahssi[9], Ouahiba Aziez[10], Camilo Bastidas[11]**

[1]Graduate Faculty of Interdisciplinary Research, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan
[2]Integrated Graduate School of Medicine, Engineering, and Agricultural Sciences, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan
[3]Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, ID, USA
[4]Division of Environmental Science and Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-ro, Pohang, Gyeongbuk 37673, Republic of Korea
[5]School of Geography, Earth and Environmental Science, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
[6]Universitas Padjadjaran, Faculty of Fisheries and Marine Science, Raya Bandung Sumedang Street KM. 21 Jatinangor, Sumedang, 45363, Indonesia
[7]Department of Civil Engineering, University of Ottawa, Ottawa K1N 6N5, Canada
[8]Enviro Solutions, Kuala Lumpur, 59200 Malaysia
[9]Faculty of Science, Ibnou Zohr University. P 8106, Agadir 80000, Morocco
[10]University of Boumerdès Faculty of Technology Frantz fanon City-Boumerdes, Algeria
[11]Faculty of Science, University of Cantabria, Avda. de los Castros s/n. 39005 Santander, Spain

*Correspondence: yrubi@yamanashi.ac.jp

**SUBMITTED: 19 January 2024; REVISED: 11 March 2024; ACCEPTED: 13 Maarch 2024**

**ABSTRACT:** Chlorpyrifos (CP) is a widely used organophosphate pesticide known for its recalcitrant nature, raising concerns about potential ecological and health impacts due to its toxicity. Many plants and animals are contaminated with this pesticide. Microbial biodegradation offers an environmentally friendly and effective method to remove CP from the environment and mitigate its impacts, especially given its low cost, particularly when bioremediation is conducted on-site. Different types of microbial species have been found to function under various environmental conditions, with some, like Pseudomonas nitroreducens PS-2 and Pseudomonas aeruginosa (NCIM 2074), showing promising results with degradation rates of up to 100%. However, challenges exist, such as partial degradation caused by the presence of metabolites, and the recalcitrant nature of CP, which can impede microbes' ability to effectively degrade its hydrocarbon ring. Overall, a combination of approaches, such as microbial and algal methods, or the discovery of new microbial strains, can help overcome these challenges and further enhance the long-term viability of this technique.

**KEYWORDS:** Chlorpyrifos; biodegradation; microbes; pesticide; bioremediation

## 1. Introduction

CP, also known as O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate, was first introduced in 1965 by Dow Chemical Company, USA. Since then, it has been extensively utilized in both agriculture and sanitation industries worldwide, significantly contributing to global food production. It ranks as the fourth most extensively used pesticide, following endosulfan, acephate, and monocrotophos [1]. This colorless to white crystalline solid effectively controls pests when applied in regulated amounts, without causing harmful effects [2]. For instance, in India, CP is primarily used to control insect pests in apples, rice, cotton, and Chinese cabbage through foliar treatment [3]. However, indiscriminate usage in activities such as crop handling, accidental spills, and container rinsing has led to excessive contamination of aquatic and soil components, raising serious environmental and human health concerns. As Sharma and Pandit describe, pesticides are chemical or biological agents that can kill or incapacitate target pests such as insects, plant pathogens, weeds, mollusks, birds, and nematodes [3].

Sharma and Pandit also highlight CP's high potential for negative impacts in occupational applications [3]. Its toxicity is relatively high, capable of adversely affecting reproductive capacity, the nervous system, cardiovascular and respiratory systems [4]. Characteristics such as bioaccumulation, high lipophilicity, long-range transport potential, and extended half-life contribute to CP's high toxicity and slow degradation [5]. Exposure to low levels of CP during pregnancy can interfere with mammalian nervous system development [6]. Furthermore, CP's metabolite, chlorpyrifos-oxon, an acetylcholinesterase inhibitor, can cause mortality and detrimental health effects in humans and potentially damage non-target organisms, as acetylcholinesterase is found in all vertebrates [7]. Due to its high toxicity, stable nature, and less soluble active compounds, other useful organisms like earthworms, bees, and spiders can also be harmed [8]. The detection of CP in human breast milk and calls for its environmental abolition from foodstuffs highlight the importance of removing CP from the environment to prevent detrimental effects on human health, especially in children [5]. Scientists and researchers are therefore prompted to seek biological and biotechnological methods to address this issue [9]. An emerging trend in CP treatment is biodegradation by microbes, a cost-effective and reliable method for safely removing the pesticide. Many reports demonstrate the capability of various bacterial species to degrade CP. Additionally, some research confirms that algae, fungi, and yeast can also break down CP.

## 2. Sources and Pathway

CP is widely available worldwide as many major agrochemical companies have historically produced and marketed chlorpyrifos-based products, such as Syngenta and Bayer CropScience. These agrochemical companies are mostly responsible for the production of CP. Thereafter, CP-based products are available for purchase at various retail stores for individuals or homeowners who have gardening or pest control needs. Therefore, CP is mostly applied to primary producers in the food chain to control pests. Crops, in particular, are the ones that CP is mostly used on since farmers account for a large percentage of the product consumer.

CP synthesis involves a multistep process with 3-methylpyridine as a starting compound, eventually reacting 3,5,6-trichloro-2-pyridinol (TCP, an intermediate formed from the partial hydrolysis of 2,6-dichloropyridine, followed by peroxide mediated chlorination) with O,O-diethyl phosphorochloridothioate and a base [10]. The formed CP product contains several functional groups that contribute to its chemical properties and potent biological activity. The key functional groups in CP include the organophosphate group, chlorine, and diethyl phosphorothioate group. The organophosphate group is responsible for the insecticidal property of CP by inhibiting the acetylcholinesterase through phosphorylation in insects [11]. Chlorine is present in the pyridinol ring in the 2,4, and 5 positions mimicking the effect of another potent chlorine-based pesticide, 2,4,5-trichlorophenol [12]. The diethyl phosphorothioate group contributes to the overall stability, structure, and effectiveness of CP. The group is similar to the active functional group of the potent insecticide “Parathion” which is currently banned due to its high toxicity towards non-target organisms [13].

CP can be used in two ways, either by spraying onto the plants or apply to the soil before the plantation starts [14]. Due to the poor solubility of CP, it quickly binds to soil particles or plants upon its application. As a result, the plants tend to accumulate CP through the absorption by the roots or to a lesser extent, on the leaves surfaces. Hence, some studies have shown the presence of CP in some common agricultural foods. CP is detected in vegetables such as cabbage, tomato, aubergine, lettuce, carrot and ladyfinger [15-18], not to mention it is also found in fruits such as pears and apples [19, 20]. To analyze the potential impact of CP and how to treat it, the movement pattern of CP in the environment must be studied. As mentioned above, a large portion of CP attaches to particles in soil or on the plants after being applied, but due to the characteristics of having a vapour pressure at around $1.9 \times 10^{-5}$ mmHg at 25°C, some CP is very likely to volatilize into the air at a fast rate upon application [21]. As a consequence, measurable amounts of CP have been reported in the atmosphere. According to Hayward et al., CP can undergo degradation faster in the atmosphere with shorter residence time [22]. In general, there is very little CP residue entering nearby water sources. CP usually volatilizes from the water surface when it manages to enter a water system. Despite that, when a higher concentration of CP is used, the amount of CP residue that enters water sources can potentially increase to an extent where it can pose a danger to the environment. Adsorption reduces CP mobility, thus increasing its persistency in the soil and preventing CP from leaching, but it also contributes to the key off-site migration route to nearby water sources when the CP is attached to the soil sediments [23]. During the migration, the contaminated soil sediments are likely to contaminate the groundwater as well. Although it is less possible for CP to flow into water bodies through surface runoff, CP can still eventually flow into the water bodies when the contaminated groundwater flows into the water bodies. The movement of CP in the environment is shown below in Figure 1.

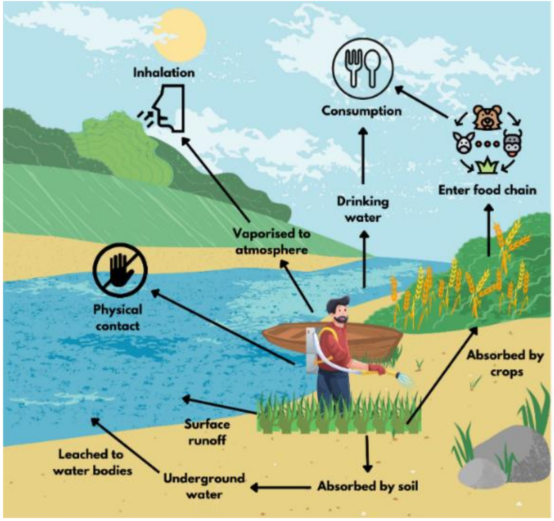

**Figure 1.** Movement of chlorpyrifos in the environment.

Many researchers have been conducting surveys to analyze and determine the CP residue inside both agricultural crops and organisms that might have accidentally ingested CP-contaminated crops or animals. Hence, some relevant studies have been referred to in this section. Due to the accumulation of CP in soil and the absorption by the plants, agricultural crops were found to contain CP, which can potentially pose a danger to whoever consumes the foods. Table 1 shows the concentration of CP found in vegetables and fruit samples.

**Table 1.** Concentrations of chlorpyrifos found in fruits and vegetables.

| Types of food | CP residues found in food samples (mg/kg) | Reference |
|---|---|---|
| Chinese kale | 0.027 | [18] |
| Cucumber | 0.146 | [18] |
| Chinese cabbage | 0.332 | [18] |
| Radish | 1.69 | [16] |
| Carrot | 2.90 | [16] |
| Cabbage | 3.02 | [16] |
| Celtuce | 3.47 | [16] |

According to these studies, celtuce is found to have the highest concentration of CP residues at 3.47 mg/kg, while Chinese kale has the lowest CP residues concentration at 0.027 mg/kg. Apart from that, as discussed earlier, CP is capable of migration into water bodies such as rivers, lakes and oceans, aquatic organisms have been found to contain CP residues in their bodies. In a study conducted by Sun and Chen, 814 samples of marketable fish were analyzed for CP residues [24].

**Table 2.** Concentrations of chlorpyrifos found in fish samples.

| Types of fish source | Number of fish | Number of detections | Maximum CP residue concentration (ng/g) | Detected CP residue concentration (ng/g) | References |
|---|---|---|---|---|---|
| Wild fish | 291 | 15 | 64 | 25 ± 23 | [24] |
| Farmed fish | 523 | 122 | 463 | 17 ± 47 | [24] |
| *Jenynsia multidentata* | 125 | - | - | Intestine: 67 ± 49 | [25] |
| | | | - | Liver: 58 ± 25 | [25] |
| | | | - | Gills: 42 ± 31 | [25] |
| *Lepomis macrochirus* | - | - | - | 3.3 | [26] |
| *Oncorhynchus mykiss* | - | - | - | 3.0 | [26] |
| *Ictalurus punctatus* | - | - | - | 13.4 | [26] |
| *Notemigonus crysoleucas* | - | - | - | 35 | [26] |

As seen from the result of the study shown in Table 3, a total of 137 samples were detected to contain CP residues, which can be interpreted as a detection rate of 17%. The farmed fish have a higher detection rate at 23%, which is 122 samples out of 523 samples, while the wild fish samples caught from the open sea have only 5% of detection rate. The highest concentration of detected CP residue is also found in the farmed fish, up to 463 ng/g. Despite that, the mean concentrations of detected CP residue also show a difference between the wild fish and farmed fish, with farmed fish showing more variation in the CP residues. Fishes exposed to CP have been observed to exhibit fin haemorrhages, and some fishes have shown convulsion with muscle contraction by the inhibition of acetylcholinesterase during the exposure [27].

## 3. Current Status and Challenge

As of March 2021, around 35 countries have banned CP, which is now listed in the PAN International consolidated list of banned pesticides [5]. Despite being outlawed in the majority of countries, CP can still be found in agricultural, soil, and water samples in some countries [28]. A ban on CP and harsh import regulations have been imposed after the European Food Safety Authority declared that ‘there is no safe level of exposure to CP' [14]. On the other hand, although the U.S. EPA proposed to ban CP back in 2015, the government rejected it in 2017, resulting in low levels of CP still being used for specific agricultural crops [29, 30]. Without strict regulations on limiting the usage of CP, CP is still found to be used in many countries. Table 3 shows the tabulated data of CP status in countries around the globe, the number and types of samples collected, the amount of CP residues detected as well as the year of detection.

**Table 3.** Detection of chlorpyrifos residues distributed in countries worldwide.

| Country | CP status in the country | Number and type of samples collected | Amount of CP residues detected in the sample | Reference |
|---|---|---|---|---|
| United States | Restricted usage | 152 cilantros | 0.002 to 0.67 mg/L | [31] |
| Brazil | Still in use | 5 randomly collected milk samples | Milk - 0.06 to 5.85 μg/L | [32] |
| Spain, Portugal | Banned | Imported organic wine collected samples | 15.96 μg/kg | [33] |
| | | 3 imported organic samples of Cayenne pepper, olive oil, and sweet potato | Olive - 9.8 μg/kg | |
| Chiang Rai, Chiang Mai, and Nan Provinces of Thailand | Banned | 160 vegetable samples | Cucumber - 275 μg/kg | [18] |
| Northern Sinaloa state, Mexico | Still in use | Buenaventura and Burrión ditches samples | Buenaventura ditch - 5.49 μg/L<br>Burrión ditch - 3.43 μg/L | [34] |
| Hyderabad, India | Banned | Vegetable samples | Presence of CP in tomato, eggplant, ladyfinger, cauliflower, and cabbage | [35] |
| Nagarpur and Saturia sub-district, Bangladesh | Still in use | 40 samples were collected from paddy field water, pond water and tube-well water | 37.3 μg/l of CP detected in one water sample | [36] |
| Balai Ringin, Sarawak, Malaysia | Still in use | Detection from topsoil surface to sub soil | 0.15 mg/kg | [37] |
| Tarat, Sarawak, Malaysia | Still in use | Detection from topsoil surface to sub soil | 1.65 mg/kg | [37] |
| Semongok, Sarawak, Malaysia | Still in use | Detection from topsoil surface to sub soil | 1.4 mg/kg | [37] |

As referred to in the table above, some countries that have banned the use of CP are found to still have CP residues in some of the products in the countries. Hence, it is impossible to completely ban and prevent the usage of CP. An environmentally friendly method of treating the CP residue is thus crucial to prevent any harmful effects from endangering both the environment and the ecosystem. There are a few challenges when it comes to managing the impacts that CP residue can bring upon both the environment and the ecosystem. The first challenge is the persistence of CP residue in the environment. The half-life of CP in the soil typically ranges from just a few days up to 4 years, and it can be greatly affected by several factors, such as the type of microorganisms present in the soil, types of soil, application rate of the pesticide, ecosystem type and weather conditions [38]. Consequently, while the residue remains in the soil for a longer period, the possibility of plants absorbing the residue and environmental contamination increases. Another challenge is the detection of CP residues. One of the most common methods of detecting CP residues is by using spectroscopy techniques. However, not only it is time-consuming and costly, but it also requires an analytical laboratory and manpower with expertise to conduct the analysis [5], making it a challenge even for the EPA. An alternative approach for detecting pesticides used in agricultural activities is hence needed.

Lastly, some CP degradation methods are also facing challenges due to some limitations. For instance, although the photocatalysis and adsorption (AOP) employing nanomaterials has shown some signs of succeeding in removing CP, a thorough investigation should be conducted on the solubility and toxicity of chemicals used for surface modification of different nanotubes, metal oxide nanoparticles and metals before the developing this technology based on these materials [5]. Other than that, another approach like enzyme-based biodegradation is also encountering some challenges, such as the loss of enzymatic activity due to physicochemical changes in the reaction environment [5].

## 4. Case study and impact

The three main types of exposure to CP are by contact, ingestion or vapour. These are also the pathways that CP can take to enter an organism's body. The oral route accounts for 70% of the CP that enter an organism among the three pathways [39, 40]. Therefore, in the following sections, the impact of CP on species and humans will be discussed. Relevant case studies from researchers are also included in order to provide data as a reference.

### *4.1. Impact on species.*

The impact of CP on organisms has been thoroughly and actively researched by scientists to analyze the potential damage it can cause when organisms are exposed to a certain concentration of CP. As an acetylcholine esterase inhibitor, CP is found to be able to interfere with the organisms' neurobehavioral development and even alter their behaviours. Since agricultural crops can absorb CP from the soil, species that feed on pesticide-laden fruits and seeds such as insects and small vertebrates that live around the farms can be impacted by the contaminated plants. As a result, the contaminants accumulate in their bodies as they continue to prey on other contaminated prey or consume the contaminated crops. Many species of birds rely on insects as their main food source, in a study conducted by Eng et al. on the white-crowned spar sparrow (*Zonotrichia leucophrys*), showed that a consumption of 8 CP granules every day for over 3 days can adversely impact the migration ability of the bird since it can cause delay and misdirection in migration, which can further results in the decline of the birds populations [41]. In a study, the zebrafish species were exposed to low and high concentrations of CP, which are 2μM and 5μM respectively. As a result, muscle exhaustion, oxidation stress and disruption of neurotransmitter metabolism were observed in the muscle of the zebrafish when exposed to the higher dosage of CP [42]. Besides that, CP is also responsible for the decline in populations of other non-target pollinators like honeybees (*Apis mellifera*) due to the oxidative stress caused by CP in the nervous system of these species [43]. Table 4 shows some effects of the toxicity of CP on several tested organisms. Not only the impact of CP is detrimental to the organisms' health or growth, but it is also lethal since some studies have shown that exposure to CP can increase species' mortality, such as rats and piglets.

**Table 4.** Impact of toxicity of chlorpyrifos on several species.

| Targeted species | Concentration of CP | Effects of toxicity | References |
|---|---|---|---|
| Freshwater fish<br>*Channa punctatus*<br>(Bloch) | 5 ppm | Effects on the gills:<br>– fusion of lamellae (LFU)<br>– swollen tip of secondary lamellae (STSL)<br>– salt cell (SC)<br>– red blood cells (RBC)<br>– necrotic lamellae (NL)<br>– lifting of lamellae epithelium (LLE)<br>– proliferation of chloride cells (PCC)<br>– mucoid metaplasia (MM)<br>– clubbed lamella (CL)<br>Effects on the liver:<br>– necrotic hepatocytes (NHC)<br>– blood conjunction (BC)<br>– vacuolation (V)<br>– portal vein (PV)<br>Effects on the intestine:<br>– severe abnormalities in the digestion process | [44] |
| Sprague-Dawley Rat | Exposure to 5300 mg/m$^3$ for 4 h | – Mortality up to 80% in male rats | [21] |
| Piglets | - | – High mortality: Cholinergic overstimulation, leads to: Dyspnoea and Diarrhoea | [21] |
| Zebrafish | 100-300 $\mu g\ L^{-1}$ | – Developmental toxicity<br>– Oxidative stress<br>– Neurotoxicity<br>– Decrease locomotor behaviour | [45] |
| Rainbow fish<br>(*Poecila reticulata)* | 0.176 ppm/L | – Exhibition of aggressive behaviour,<br>– Rapid gulping of water,<br>– Increased opercular movement<br>– Abnormal and erratic swimming movements | [26] |

### *4.2. Impact on human.*

While the toxicity impact of CP on organisms has been found to be dangerous and lethal, the severity is also similar when it comes to exposure of CP to human. In humans, apart from being a skin and eye irritant, some other symptoms of exposure to CP are blurred vision, numbness, nausea, incoordination, headache, dizziness, abdominal cramps, tremor, tingling sensations, difficulty breathing or respiratory depression, slow heartbeat [46]. As the dosage of exposure increases, severe results such as unconsciousness, incontinence, convulsions or fatality can also occur [21]. Alterations in the thyroid and adrenal glands are one of the results of CP contamination, which can further result in reducing serum levels of the corresponding hormones [38]. Moreover, children are susceptible to suffering from pervasive developmental disorder issues, attention deficit or hyperactivity disorder, and psychomotor and mental development index delays when being exposed to a larger concentration of CP [47]. Besides that, CP is also identified and proven to be anti-androgenic [46, 47] and estrogenic [48, 49]. This is associated with reproductive issues such as birth weight and length problems, not to mention damage of DNA in sperm concentration, sperm motility, cervical fluid, cord blood, meconium, and breast milk [50]. Despite some studies stating that CP is only considered moderately toxic to humans as it mainly attacks the nervous system by inhibiting cholinesterase, which is an enzyme that is crucial for nervous functions [46], it has been found that CP can cause inhibition of DNA synthesis, interference with gene transcription, neural disorders, altered function of neurotrophin signaling cascade and synaptic function [51]. Ultimately, long-term assessments must be conducted to better evaluate the toxicity and behavioural effects of CP so that its impact on both species and humans can be identified.

## 5. Microbial Biodegradation

When it comes to types of treatment methods for degrading or removing CP from the environment, there are a variety of them. A great number of physical, chemical and biological methods such as oxidation, membrane filtration and adsorption have been the conservative methods for treating CP, especially removing the ones from soil and water [52]. Chemically, CP degradation mostly involves hydrolysis reactions targeting the C─O linkage between the ring and side chain producing diethylthiophosphoric acid (DETP) and TCP. Further transformation of the TCP ring through ring breakage leads to complete detoxification, rendering the intermediates less toxic compared to the original chlorpyrifos [53]. The efficiency of CP degradation depends on pH and was observed to be around neutral to slightly basic conditions.Nonetheless, those methods tend to have some downsides such as the pre-sampling process, instrumental methods of analysis and some chemical processes that are time-consuming and labour-intensive [54]. Therefore, creative and innovative methods are needed to overcome the limits of conventional treatment technology. A viable method is to biodegrade CP using microbes, also known as microbial biodegradation, which is an eco-friendly, efficient and cost-effective way of removing the pesticide [3]. In this process, the microbes can consume the pesticides along with other materials co-metabolites of food or energy so that the toxic waste can be used as a carbon or energy source [3]. Several bacteria and fungi have been identified to be capable of degrading CP either catabolically or co-metabolically, including bacterial species in the genera *Bacillus*, *Pseudomonas*, *Arthrobacter,* and *Micrococcus* [5, 38]. This is because organophosphate degrading enzymes, a bacterial enzyme that can break down a variety of neurotoxic organophosphorus pesticides are found in these microorganisms, such as the Parathion hydrolases (OPH) found in the *Flavobacterium* species [3]. Nonetheless, some factors can affect the effectiveness of OPH activities in the microbes. These factors include temperature, carbon sources, pH, incubation time, metal ions and the presence of some other chemical compounds. When these factors are well controlled, the OPH activities can be maximized to obtain the best results. For instance, for species such as *Pseudomonas stutzeri* S7B4 and *Pseudomonas aeruginosa* NL01, the optimum temperature for the OPH activities are 35°C and 37°C [55, 56].

### *5.1. Bacteria.*

A variety of bacteria species have been proven to be able to biodegrade CP, such as the *Bacillus*, *Burkholderia*, *Arthrobacter*, *Flavobacterium*, *Azotobacter* and *Pseudomonas* [5]. Different species can result in the ability to biodegrade different concentration of CP, different time taken to completely biodegrade CP, as well as the rate of degradation. Some details of the biodegradation by some bacteria are shown in Table 5 below.

**Table 5**. Biodegradation of chlorpyrifos by different bacteria species.

| Bacteria species | Degradation (%) | Duration (hours) | Medium of contact | Concentration (mg/L) | References |
|---|---|---|---|---|---|
| *Pseudomonas diminuta* | 12 | - | - | - | [57] |
| *Pseudomonas aeruginosa* | 92 | 720 | Soil | 50 | [58] |
| *Pseudomonas sutzeri* | 88 | 192 | - | - | [59] |
| *Pseudomonas fluorescence* | 43 | 240 | - | - | [51, 58] |
| *Pseudomonas putida* MAS-1 | 90 | 24 | Mineral salt | 2000 | [60] |
| *Pseudomonas nitroreducens* PS-2 | 100 | 672 | Rhizospheric soil of ryegrass | - | [61] |
| *Pseudomonas aeruginosa* (NCIM 2074) | 100 | 168 | - | 50 | [62] |
| *Pseudomonas fluorescence* | 43 | 240 | - | - | [51, 58] |
| *Pseudomonas sp.* (iso 1) | 91 | 312 | - | - | [63] |
| *Pseudomonas aeruginosa* | 92 | 720 | Soil | 50 | [58] |
| *Pseudomonas stutzeri* B-CP5 | - | 168 | Soil | 300 | [64] |
| *Cellulomonas fimi* | 100 | 72 | - | 50 | [11] |
| *Bacillus pumilus* | 89 | 336 | - | 1000 | [65] |
| *Sphingobacterium sp.* JAS3 | 100 | 120 | Soil | 300 | [66] |
| *Stenotrophomonas sp.* G1 | 42.6 | 20 | Plant sludge | 50 | [67] |
| *Ochrobactrum sp.* JAS2 | 100 | 12 | Soil | 300 | [68] |
| *Acinetobacter calcoaceticus* | 100 | 1008 | Soil | 100 | [69] |

### *5.2. Fungi.*

Besides bacteria, fungi are also found to be able to effectively biodegrade CP residue found in the environment by introducing minor structural changes to CP, making it to be more susceptible to further biodegradation by other bacteria [70]. This is because the high tolerance of fungal strains can effectively biodegrade high concentrations of pesticide when compared to other microbes like bacteria, [5]. Although the usage of fungi as microbes for biodegradation has not received much attention, studies are showing the benefits of using fungi when bacteria or other microbes have failed in biodegrading pesticides [5]. **Table 6** below shows the biodegradation of CP by different species of fungi.

**Table 6.** Biodegradation of chlorpyrifos by different fungi species.

| Fungi species | Degradation (%) | Duration (hours) | Medium of contact | Concentration (mg/L) | References |
|---|---|---|---|---|---|
| *Acremonium sp.* GFRC-1 | 83.90 | 480 | Soil | 300 | [71] |
| *Aspergillus sp., Penicillium sp., Eurotium sp., Emericella sp.* | 69.4–89.8 | 168 | Soil | 25–200 | [72] |
| *Cladosporium cladosporioides* Hu-01 | >90 | 120 | Soil | 50 | [73] |
| *Ganoderma sp.* JAS4 | 100 | 24 | Soil | 300 | [74] |
| *Phanerochaete chrysosporium* | 100 | 144 | - | 50 | [11] |

As seen from the table, fungi species *Phanerochaete chrysosporium* and *Ganoderma sp.* JAS4 are capable of reaching 100% of degradation, not mention that the latter can ahieve the results in under 24 hours. Other than that, some fungi such as *Flammulina velupites, Avatha discolor* and *Dichomitus squalens* have also been observed to be able to degrade a variety of pesticide apart from just CP like triazine, dicarboximide and phenylurea [8]. *Cladosporium cladosporioides* Hu-01 have shown the ability to simultaneously degrade the original chlorpyrifos and the intermediate TCP through first-order model degradation kinetics. The optimal conditions of chlorpyrifos degradation using strain Hu-01 are pH 6.5, 26.8°C for 4.7 days [73].

### *5.3. Advantages and disadvantages of microbial biodegradation.*

The first advantage of using microbes to biodegrade toxic pesticides like CP is environmentally friendly. This is because microbial biodegradation has always been a part of nature, in which organic molecules in the environment are reduced to simpler compounds, mineralized, and redistributed throughout the environment via elemental cycles such as the carbon, nitrogen, and sulfur cycles [75]. In a study conducted by Anwar et al., B. pumilus strain C2A1 was found to be capable of biodegrading CP across different values of pH to as low as pH 5.5, not to mention that its tolerance for CP can be as high as 1000 mg/L [66]. Therefore, this advantage enables the microbe to be used in a variety of environments. Besides that, engineered P. putida MB285 cells are also found to be able to degrade CP completely from pH values ranging from 2 to 7 and temperature ranging from 5 to 55 °C [76]. Even though that the optimum pH and temperature for the reaction are 3.0 and 25 °C respectively [76], it is undeniable that this microbe can also work under different environmental conditions. Other than that, as shown in Table 5, Pseudomonas putida MAS-1 can degrade CP up to 2000 mg/L. On top of that, the rate of degradation can be up to 100% for certain species such as Pseudomonas aeruginosa (NCIM 2074) and Pseudomonas nitroreducens PS-2. Moreover, high efficiency and cost-effectiveness have always been the advantages of biodegrading toxic waste using microbes [3]. Provided that the ideal conditions for the process are present, the process of bioremediation can be less costly when it is being done on-site, not to mention the site disruption can be minimized [8]. In addition, when coupled with additional physical or chemical treatments, it is possible to completely remove the toxic pesticide, which remove long-term liabilities, hence resulting in higher public acceptance [8].

Despite all the advantages mentioned above, there are still some disadvantages to consider for this method of removing CP from the environment. One of the disadvantages of using this method is the required time to complete degradation process. As seen from Table 5 above, the duration of the biodegradation can last from 24 to as long as 700 hours. Most of the Pseudomonas species take more than 100 hours for the process to complete. Hence, being a time-consuming method can result in higher overall treatment cost and less effective especially when it comes to treating a larger amount of CP as compared to other alternatives such as photodegradation or using activated carbon as an adsorbent to remove the pesticide [8, 77]. Moreover, as mentioned above, different environmental parameters are required by different types of bacteria. This can make the biodegradation process harder to maintain when multiple bacteria are to be used simultaneously to remove CP from the environment.

On the other hand, the microbes can potentially develop resistance against the pesticide, hence affecting the long-term reliability of this method. Pesticides can activate the efflux pumps, the inhibition of the microbes' outer membrane pores for resistance to antibiotics, and lead to gene mutations, which ultimately cause the microbes to develop antibiotic resistance [78]. In a study conducted by Ramakrishnan et al., it was found that high concentration of pesticide has nearly no effect on the microorganisms, however, In pesticide-degrading bacteria, cross-resistance to antimicrobial agents is very likely [79]. The microbes tested include Escherichia coli O157:H7 and Vibrio phosphoreum, which were tested on CP [79]. Ultimately, the development of antibiotic resistance in the microbes can disable their abilities to biodegrade CP, hence negatively affecting the effectiveness and efficiency of the biodegradation process.

## 6. Future Research and Prospect

With all being said, although microbial biodegradation has many advantages and potential to bioremediate the issues of pesticides residues in the environment, there are still challenges to be overcome to further improve this method to be more effective and reliable so that it can be utilized worldwide. Since this method is mainly used in soil environment, factors such as the temperature and pH value can affect the effectiveness of the biodegradation. Hence, partial degradation is not uncommon when treating CP in different environments, which can form and accumulate metabolites in the soil system [8]. The metabolites can sometimes be toxic and harder to dissolve than the parent compounds, thus further decreasing the rate of pesticide degradation [8]. A potential solution to this issue is searching for electro-active bacteria that can biodegrade the pesticide. The biodegradation rate can be vastly increased by the enhanced removal of the metabolites due to the presence of electrodes [80]. Besides that, since CP is a recalcitrant organic compound, the anionic species found in the compound can limit the biodegradation by the microbes. Anions such as sulfates and chlorides tend to form a strong bond with the hydrocarbon ring, hence, the increased toxicity of the anions may prevent the microbes from attacking the ring structure of the pesticides, resulting in less effective biodegradation [8]. Moreover, there are also other limitations to this technique such as the inability to complete the mineralization of the pesticides and the lack of efficiency in biodegrading complex pesticide mixtures, especially in heavily polluted places [81]. Therefore, it is recommended to thoroughly research the mixtures or compounds that may be mixed with CP before the biodegradation commences.

## 7. Conclusion

CP, a recalcitrant organic compound, persists in the environment for extended periods due to its resistance to natural degradation. Microbial biodegradation shows promise in removing CP and mitigating its impacts, though the development of resistance poses challenges. Solutions like genetically modified microorganisms, such as electro-active bacteria, can address this issue by removing CP metabolites. Additionally, thorough studies on solubility and toxicity of reaction compounds are recommended. Combining approaches like microbial and algal methods or utilizing new microbial strains with unique traits may enhance efficacy across diverse environmental conditions.

## Acknowledgments

The authors would like to acknowledge the team of University of Yamanashi Japan, University of Idaho USA, POSTECH Republic of Korea, University of Birmingham United Kingdom, Universitas Padjadjaran Indonesia, University of Ottawa Canada, Ibnou Zohr University Morocco, University of Boumerdès Algeria, University of Cantabria Spain for the technical support in this project.

## Competing Interest

The authors declare no conflict of interest.

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