Environmental Science and Engineering

Shree Nath Singh *Editor*

Microbe-Induced Degradation of Pesticides

Environmental Science and Engineering

Environmental Science

Series editors

Ulrich Förstner, Hamburg, Germany
Wim H. Rulkens, Wageningen, The Netherlands
Wim Salomons, Haren, The Netherlands

More information about this series at http://www.springer.com/series/3234

Shree Nath Singh
Editor

Microbe-Induced Degradation of Pesticides

Editor
Shree Nath Singh
CSIR-National Botanical Research Institute
Lucknow, Uttar Pradesh
India

ISSN 1863-5520 ISSN 1863-5539 (electronic)
Environmental Science and Engineering
ISSN 1431-6250
Environmental Science
ISBN 978-3-319-45155-8 ISBN 978-3-319-45156-5 (eBook)
DOI 10.1007/978-3-319-45156-5

Library of Congress Control Number: 2016948230

© Springer International Publishing Switzerland 2017
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To parents in heavenly abode

Preface

Pesticides are usually referred as a broad range of insecticides, fungicides and herbicides. Presently, there are 900 pesticide products and 600 active pesticide ingredients available in the market. Although millions of tonnes of pesticides are applied in the agriculture and horticulture, less than 5 % of pesticides only reach to the targeted organisms and rest gets deposited on the soil and non-targeted organisms and also moves to water bodies and the atmosphere. The fate of these pesticides is governed by the abiotic factors (temperature, moisture, soil, pH, etc.) as well as biological and chemical reactors. Abiotic degradation of pesticides is mediated by oxidation, reduction, hydrolysis and photolysis and rearrangement, while biotic degradation is caused by both microbial communities (bacteria, fungi, etc.) and plant species.

In view of the above facts, the editor has compiled the latest developments on biodegradation of chemical pesticides used in agriculture in this edited volume contributed by Indian and foreign scientists which will serve as a ready reckoner not only to scientists, but also to policymakers, teachers, students and the farmers.

In this endeavour, I would like to thank all the contributors for their positive response and active participation by contributing the latest updates on the degradation of different chemical pesticides. I would like to thank my research scholars Ms. Nitanshi Jauhari and Mrs. Shweta Mishra for their academic and technical support. Besides, untiring support by Mr. Dilip Kumar Chakraborty in preparing the book manuscript is heartily acknowledged.

Lastly, I would like to thank my family members: Mrs. Manorama Singh (wife), Ragini (daughter) and her kids Antra and Avantika and Pritish (son) and Vishali (daughter-in-law) for their inspiration, endurance and moral support in this endeavour.

Lucknow, India — Shree Nath Singh

Contents

Microbe-Assisted Degradation of Aldrin and Dieldrin 1
Adi Setyo Purnomo

Microbial Degradation of Chlorophenols 23
Muftah H. El-Naas, Hussein A. Mousa and Maisa El Gamal

Remediation of Endosulfan Contaminated System by Microbes 59
Mathava Kumar and Ligy Philip

Bioremediation of Isoproturon Herbicide in Agricultural Soils 83
Sabir Hussain, Tanvir Shahzad, Muhammad Imran, Azeem Khalid
and Muhammad Arshad

Endosulfan a Cyclodiene Organochlorine Pesticide: Possible Pathways of Its Biodegradation 105
Dileep K. Singh and N. Sarat Singh

Fungal Degradation of Organochlorine Pesticides 131
Héctor Hugo León-Santiesteban and Refugio Rodríguez-Vázquez

Microbial Degradation of Endsulfan and Endsulfan Sulfate 151
Ryuichiro Kondo

Microbe Induced Degradation of Pesticides in Agricultural Soils 167
Durgesh Kumar Jaiswal, Jay Prakash Verma and Janardan Yadav

An Overview on Microbial Degradation of Lindane 191
J.M. Saez, A. Alvarez, M.S. Fuentes, M.J. Amoroso and C.S. Benimeli

Degradation of Atrazine by Plants and Microbes 213
S.N. Singh and Nitanshi Jauhari

Index 227

Contributors

A. Alvarez Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina; Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina

M.J. Amoroso Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina; Universidad del Norte Santo Tomás de Aquino, Tucumán, Argentina; Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina

Muhammad Arshad Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

C.S. Benimeli Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina; Universidad del Norte Santo Tomás de Aquino, Tucumán, Argentina

Muftah H. El-Naas Gas Processing Center, College of Engineering, Qatar University, Doha, Qatar

M.S. Fuentes Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina

Maisa El Gamal Chemical and Petroleum Engineering Department, UAE University, Al-Ain, United Arab Emirates

Sabir Hussain Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Muhammad Imran Department of Soil Science, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan; Environmental Microbiology, Soil Science Division, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan

Durgesh Kumar Jaiswal Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Nitanshi Jauhari Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow, India

Azeem Khalid Department of Environmental Sciences, PMAS University of Arid Agriculture, Rawalpindi, Pakistan

Ryuichiro Kondo Kyushu University, Fukuoka, Japan

Mathava Kumar Environmental and Water Resources Engineering Division, Department of Civil Engineering, Indian Institute of Technology Madras, Chennai, India

Héctor Hugo León-Santiesteban Departamento de Biotecnología Y Bioingeniería, Centro de Investigación Y de Estudios Avanzados Del Instituto Politécnico Nacional, Mexico City, Mexico

Hussein A. Mousa Chemical and Petroleum Engineering Department, UAE University, Al-Ain, United Arab Emirates

Ligy Philip Environmental and Water Resources Engineering Division, Department of Civil Engineering, Indian Institute of Technology Madras, Chennai, India

Adi Setyo Purnomo Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember (ITS), Surabaya, Indonesia

Refugio Rodríguez-Vázquez Departamento de Biotecnología Y Bioingeniería, Centro de Investigación Y de Estudios Avanzados Del Instituto Politécnico Nacional, Mexico City, Mexico

J.M. Saez Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina

N. Sarat Singh Department of Zoology, University of Delhi, Delhi, India

Tanvir Shahzad Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Dileep K. Singh Department of Zoology, University of Delhi, Delhi, India

S.N. Singh Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow, India

Jay Prakash Verma Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Janardan Yadav Department of Soil Science and Agricultural Chemistry, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Microbe-Assisted Degradation of Aldrin and Dieldrin

Adi Setyo Purnomo

1 Introduction

Environmental pollution is an inseparable evil associated with anthropogenic activities. Increasing human needs resulted in the growth of the industries which produce new products through modern technologies. Among the various kinds of environmental issues, synthetic pesticides, produced in the agricultural industry have become a serious environmental problem. Among the chemical pesticides, aldrin and dieldrin are chlorinated cyclodiene pesticides which are classified as persistent organic pollutants (POPs) that cause serious environmental problems. They are highly ecotoxic to higher organisms, because of their low solubility, their tendency to partition into the lipophilic phase, and also contain chlorine atoms (Foght et al. 2001). They cause numerous negative effects, including disruption of the endocrine system in birds and mammals, impairment of male reproductive ability, interference with sex hormones, eggshell thinning, and a carcinogen for humanbeings (WHO 1989). As a result of their chemical stability and lipophilicity, aldrin and dieldrin are extremely persistent in the soil and sediment environments, with a half-life of 1 year or more. Although these compounds have been prohibited over the past decades in most countries around the world, they are still found in the environment, especially in the soil in agricultural fields. In 2001, more than 100 countries signed the Stockholm Convention on Persistent Organic Pollutants (POPs), committing to eliminate the use of the 12 POPs of greatest concern, including aldrin and dieldrin (Xiao et al. 2011).

The removal of pollutions from contaminated waters and soils has become an environmental priority, and both physicochemical and biological remediation pro-

A.S. Purnomo (✉)
Department of Chemistry, Faculty of Mathematics and Natural Sciences,
Institut Teknologi Sepuluh Nopember (ITS), Kampus ITS Sukolilo,
Surabaya 60111, Indonesia
e-mail: adi_setyo@chem.its.ac.id; adi.spurnomo@yahoo.com

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_1

cesses have been studied. Although chemical and physical treatments are more rapid than biological treatments, they are generally destructive and intrusive to affected soils, energy intensive and also more expensive than bioremediation (Foght et al. 2001). Biodegradation using microorganisms, including bacteria and fungi, has been found to be a cost-effective method of treating various pollutants including aldrin and dieldrin, which has been existing in the environment since late 1960s.

2 Microbial Degradation of Aldrin and Dieldrin

For several years, dieldrin residues were assumed to appear as a result of microbial epoxidation of aldrin in soil in areas, where dieldrin itself had never been used. The indirect evidence for the conversion of aldrin to dieldrin by epoxidation was provided by Lichtenstein and Schulz (1960). Aldrin was rapidly converted to dieldrin in non-sterile soil, while little conversion occurred in the sterile soil. Aldrin also disappeared more quickly in moist soil than in dry soil, as microbes are more active in moist soil. Subsequently, Lichtenstein et al. (1963, 1965) showed that the conversion of aldrin to dieldrin was inhibited by methylenedioxyphenyl synergists (sesamex, piperonyl cyelonene, piperonyl butoxide, sulfoxide and n-propyl isome). Sesamex was the best inhibitor for the conversion of aldrin to dieldrin compared to other methylenedioxyphenyl compounds. Sesamex was also found to reduce populations of microorganisms when added to pure cultures, soil suspension, or soils, which caused no dieldrin formation. A breakdown product of sesamex, i.e. sesamol, which is also known as antioxidant, caused no significant inhibition of dieldrin formation. It indicated that the conversion of aldrin to dieldrin in soil was inhibited by synergists at relatively high rates, due to their high toxicity to microorganism populations.

Many studies have shown the microbial transformation of aldrin and dieldrin to intermediate metabolites under aerobic conditions, but the metabolic pathways are still unclear. The investigation on biodegradation of aldrin by pure cultures of soil microorganisms had been reported by Tu et al. (1968). Ninety-two pure cultures of soil microorganism were screened for degrading aldrin of which a majority showed some ability for converting aldrin to dieldrin by epoxidation. Among the fungi, *Trichoderma, Fusarium,* and *Pinicillium* were the most active for aldrin transformation. Besides, *Actinomycetes* were also effective converters, with one exception, *Bacillus* sp. which was of less importance. *Fusarium* sp., the most active isolate, converted 9.2 % of the added aldrin to dieldrin during 6 weeks of incubation period.

In some instances, the production of dieldrin was linear in relation to time. Besides, some microorganisms transformed aldrin to products other than dieldrin. Moreover, dieldrin had been transformed into 6,7-trans-dihydroxydihydroaldrin (trans-aldrin diol), photodieldrin, and ketoaldrin (Matsumura and Boush 1967, 1968; Matsumura et al. 1970; Patil et al. 1970).

The ability to epoxidize aldrin to dieldrin is obviously a common trait widely distributed among soil bacteria. Ferguson and Korte (1977) have described a number of strains of gram-positive and gram-negative soil bacteria which produce

exclusively the exoisomer of dieldrin. Twenty-two strains of soil bacteria, including representatives of the genera *Bacillus, Micromonospora, Mycobacterium, Nocardia, Streptomyces, Thermoactinomyces* and *Pseudomonas* were found to degrade aldrin to its epoxide dieldrin. Previously, Tu et al. (1968) reported production of dieldrin in 30 g-positive isolates out of 45 tested, whereas Patil et al. (1970) found 11 g-negative and gram-positive soil bacteria with this trait.

Maule et al. (1987) reported that anaerobic microbial populations, developed from soil, freshwater mud, sheep rumen, and chicken litter, could transform dieldrin to monodechlorinated products. The freshwater mud microbial population was the most effective, and transformed 96 % dieldrin to the *syn*- and *anti*- monodechlorinated products in a culture, initially containing 10 μg ml^{-1} of dieldrin approximately for 7 days. Three isolates from this culture, classified as the genus *Clostridium*, were capable of dieldrin dehalogenation, although the dehalogenation rate by each isolate was much less than by the parent population. *Clostridium bifermentans*, *Clostridium glycolium*, and *Clostridium* sp. required 54, 87 and 95 days, respectively, to transform 80 % of the dieldrin in a culture.

On the other hand, degradation of aldrin and dieldrin was also achieved in free cell cultures of *Pseudomonas fluorescens* to the level of 94.8 % for initial concentration of 10 mg L^{-1} (Bandala et al. 2006). Sakakibara et al. (2011) also reported that strain KSF27 converted dieldrin to aldrin dicarboxyric acid via aldrin trans-diol (Fig. 1). Strain KSF27 exhibited a high sequence similarity to *Pseudonocardia* spp. Based upon the genetical and morphological characteristics, strain KSF27 was found to be a new species of the genus *Pseudonocardia*, designated as *Pseudonocardia* sp. strain KSF27. Although the endo-isomer of dieldrin is less stable than the exo-isomer, there is no reason to expect microbial epoxidation to lead to exo-dieldrin in every case. In fact, exo-isomer is only produced in dissimilar genera, such as *Pseudomonas*, *Bacillus*, and the members of *Actinomycetes*.

Recently, white rot fungi (WRF) was found to degrade lignin, a complex high-molecular-mass aromatic polymer, as well as a wide spectrum of recalcitrant organopollutans, including biphenyls, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (Kamei et al. 2010). WRF are generally more tolerant to high concentrations than bacteria (Xiao et al. 2011). Xiao et al. (2011) reported that 20 white rot fungi belonging to genus *Phlebia* were investigated for their ability to degrade dieldrin. In that experiment, screening of fungi was done with 50 μL (5 mmol L^{-1}) of dieldrin. Based on the screening results, *Phlebia acanthocystis*, *Phlebia brevispora*, and *Phlebia aurea* was evaluated for their degradation capacity and metabolic products of dieldrin and aldrin degradation.

As evident from Table 1 the degradation ability of three *Phlebia* fungi was to remove over 50 % of dieldrin in a low nitrogen (LN) medium, after 42-d of incubation. Three hydroxylated products were detected as metabolites of dieldrin, including 9-hydroxyaldrin and two carboxylic acid products. It suggested that in *Phlebia* strains, hydroxylation reaction might play an important role in the metabolism of dieldrin, in which methylene moiety of dieldrin molecules might be prone to enzymatic attack by white rot fungi.

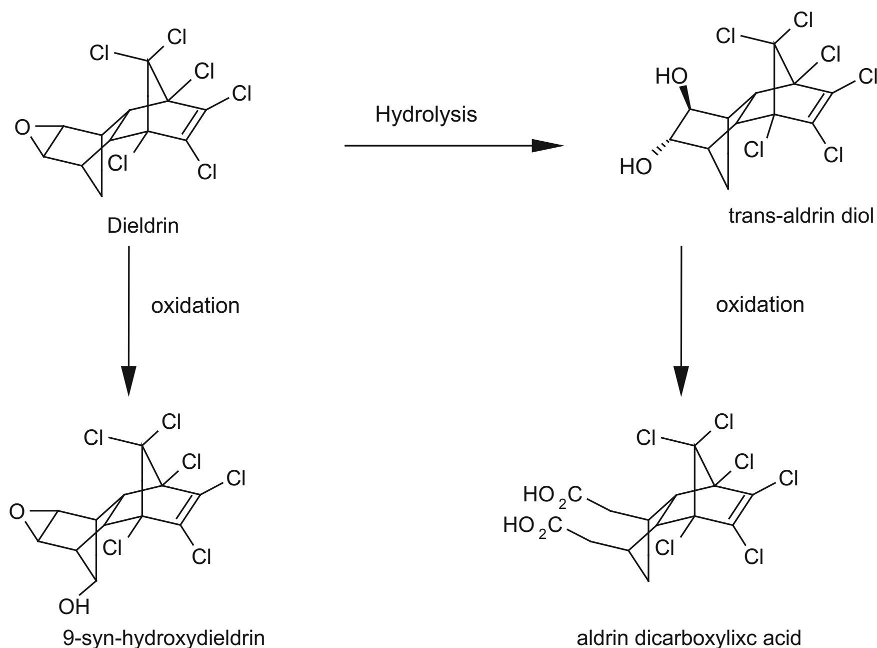


Fig. 1 Proposed metabolic pathway of dieldrin transformation by strain KSF27 (Sakakibara et al. 2011)

Kataoka et al. (2010) reported that an aerobic dieldrin-degrading fungus, *Mucor racemosus* strain DDF was isolated from a soil to which endosulfan had been annually applied for more than 10 years until 2008. Strain DDF degraded dieldrin to 1.01 mM from 14.3 mM during 10 days incubation at 25 °C. Approximately, 0.15 mM (9 %) of aldrin trans-diol was generated from the dieldrin degradation after a 1 day incubation. The degradation of dieldrin by strain DDF was detected over a broad range of pH and concentrations of glucose and nitrogen sources.

Kamei et al. (2010) reported thirty-four isolates of wood-rotting fungi were investigated for their ability to degrade dieldrin. Among these fungal isolates, *Phlebia* sp. YK543 degraded 20 % of dieldrin during the initial 7 days and then

Table 1 Degradation rate of aldrin and dieldrin by *Phlebia* fungi in low-nitrogen (LN) medium during 42-d incubation period

Phlebia fungi	Substrate	Degradation (%)
P. acanthocystis	Aldrin	96.0
	Dieldrin	56.0
P. brevispora	Aldrin	97.6
	Dieldrin	51.6
P. aurea	Aldrin	96.4
	Dieldrin	54.0

39.1 % of dieldrin during 30 days of incubation period in LN medium. 9-Hydroxylation was detected as a metabolite in the cultures of *Phlebia* sp. YK543.

On the other hand, Birolli et al. (2015) isolated marine-derived fungi *Aspergillus sydowii* CBMAI 935, *A. sydowii* CBMAI 933, *Penicillium miczynskii* CBMAI 930 and *Trichoderma sp*. CBMAAI 932 from the marine sponges *Geodia corticostylifera* and *Chelonaplysylla erecta*. In degradation studies, *P. miczynskii* CBMAI 930 showed the highest tolerance to dieldrin and catalyzed the biotransformation of dieldrin (50 mg L^{-1}) with high conversion rates (90 %) after 14 days in liquid medium. The organochlorine compounds were identified in the biodegradation reaction as endrin, endrin ketone and cyclopentene.

Matsumura and Boush (1968) reported that *Trichodermaviride*, isolated from soil heavily contaminated with various insecticides, had the ability to degrade dieldrin in liquid medium after 30 days of incubation without shaking and identified aldrin, dieldrin aldehyde, ketoaldrin, and photoisomer of ketoaldrin as metabolic products of the degradation of dieldrin in soil by microorganisms. Yamazaki et al. (2014) observed that *Mucor racemosus* strain DDF could decrease dieldrin levels with simultaneous production of a small amount of aldrin-trans-diol. The degradation was performed by adding 50 μL of an aldrin-trans-diol stock solution (1000 mg L^{-1}) and incubated for 14 days at 25 °C in the absence of light.

3 Involvement of Enzymes in the Degradation Process

Organochlorine pesticides (OCPs) act as prooxidant stressors and increase the intracellular generation of reactive oxygen species (ROS) and oxidative conditions, which, in turn, modulate levels and function of antioxidants enzymes (Osburn and Kensler 2008). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the first line of defence against ROS and other free radicals while glutathione S-transferases (GSTs) are phase II enzymes providing defence against the toxicity cause by ROS. The cytochrome P450 dependent monooxygenase (MO) and glutathione S-transferase (GST) are among the most widely studied enzymes involved in the metabolism of xenobiotics (Jensen et al. 1991). Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase in the form of serum are capable of degrading aldrin and dieldrin found in the animal body (Luckens and Phelps 1969).

In the rat, dieldrin is metabolized through the three different degradation routes. Judging from the *in vitro* studies alone, the major metabolite of dieldrin, produced in the liver, is conjugated with glucuronic acid. This metabolite is probably excreted through urine and feaces mediated by β-glucorinadase (Matthews and Matsumura 1969). Aldrin and dieldrin are substrates specific to purified armyworm midgut enzyme epoxide hydrolase. The purified armyworm enzyme is able to hydrate mono-substituted epoxides faster than 1,2-disubtituted epoxides and these, in turn,

are cleaved faster than trisubstituted substrates. Active site of enzyme may be located within restricted pit or cleft in the enzyme surface which allows ready access to the monosubstituted epoxides. However, cis-1,2-disubstituted epoxides, which remained unhindered, imposed increasingly severe steric constrains on the ingress of more bulky substrates. Perhaps, only those substrates substitute one side of oxine ring having sufficient conformational elasticity to gain access to the depression and may interact successfully with active site. Strict regioselectivity has been established for the catalytic center of rat liver epoxide hydrolase (Mullin and Wilkinson 1980). The present investigation indicates that any alteration in the activities of acid phosphatase (ACPase) and alkaline phosphatase (ALPase) creates disturbances in the normal functioning of the various tissues of *M. monoceros* after addition of aldrin, an organochlorine insecticide (Reddy and Jayaprada 1991).

Since microsomonal aldrin epoxidase of the strain housefly was not due to a single chromosome, the involvement of a regulatory mechanism was considered. It was reasoned that if the substrains were treated with an inducing agent, the substrains carry the structural gene for high epoxidase activity as the Fe parent. Heptachlor was used instead of aldrin as the enzyme substrate in order to learn whether the substrains differed in their epoxidase activities toward the two cyclodienes. Epoxidase activities of all the substrain were increased five to seven folds by treatment with phenobarbital, but none reached the level of induced Fe strain. It was interesting to note that the amount of induced enzyme in the parent strain was greater than that in any of the substrain (Schonbord et al. 1973).

The effect of cyclodiene insecticides on O-demethylase activity was studied in two strains of houseflies. An increase in microsomonal demethylase activity was observed when houseflies were treated with aldrin, dieldrin, heptachlor or heptachlor epoxide at the doses required for maximum induction of epoxide (Yu and Terriere 1972).

A reliable, cheap and highly sensitive biosensor has been developed for the near-real time monitoring of aldrin and dieldrin. The basic sensor operating mechanism relies on the inhibition of acetylcholinessterase (AchE) enzyme by organophosphate and carbamate pesticides. The degree of enzyme inhibition, which is related to the amount of pesticide present, is determined potentiometrically by measuring changes in proton activity in the locality of the enzyme immobilized working electrode. The unique characteristic of the sensor resides in the fact that stable and repeatable enzyme immobilization onto the indicator electrode was achieved within a polyacrylamide gel matrix by in situ polymerization. This novel technique gives consistent results with good correlation with samples of known concentration and provides a novel, generic approach to reliable immobilization of labile enzyme and other biological molecules. This sensor may be used in the future for the direct determination of pesticides in real samples, such as soil extracts, horticultural produce, milk and potable water, as well as for more general environmental and Hazard Analysis and Critical Control Point (HACCP) applications.

Monooxygenase activities and the corresponding half-lives of foreign compounds metabolized by this system have been studied in a range of vertebrata.

A similar relationship was found between plasma antipyrine half-lives in the dog and rabbit and the corresponding hepatic microsomonal monooxygenase activities (antipyrine as substrate with the dog, aniline and ethyl morphine with rabbit). Since most liposoluble foreign compounds must be metabolized before they can be excreted to an extent, excretion rates as well as half-lives may be related to the activity of monooxygenase and/or detoxifying enzymes. It seems likely that the rate of metabolism will be important in determining the excretion rate where conversion is slow, assuming that availability is not a limiting factor in the case of the organochlorine insecticide i.e. dieldrin. Animals, which are deficient in monooxygenase or other key detoxifying enzyme, should be identified, and should receive close attention in monitoring program for pollutants. A pesticide is likely to be efficiently accumulated by species that are deficient in the detoxifying enzymes responsible for its degradation. Finally, this approach is also helpful in the extrapolation of toxicological data to man. It can indicate which species are most likely to resemble man with regard to the metabolism of particular drug (Walker 1978). Undoubtedly, cytochrome P450 monooxygenase plays some roles in the metabolism of organochlorine pesticide, such as dieldrin and aldrin (Xiao et al. 2011).

4 Mechanism and Pathways of Degradation

Although metabolism of dieldrin and aldrin by some microorganisms has been reported, the detailed metabolic pathways were not still well understood. A number of studies have described the metabolic products of dieldrin degraded by bacteria and fungi.

A new dieldrin-degrading bacterium was isolated from the soil. Sakakibara et al. (2011) reported that *Pseudonocardia* spp. strain KSF27 converted dieldrin to aldrin dicarboxyric acid via aldrin trans-diol (Fig. 1). Although the endo-isomer of dieldrin is less stable than the exo-isomer, there is no reason to expect microbial epoxidation to lead to exo-dieldrin in every case. The exo-isomer is only produced in different genera such as *Pseudomonas*, *Bacillus*, and the members of *Actinomycetes*. The proposed pathway of metabolism of dieldrin via aldrin *trans*-diol has been shown in Fig. 1 (Sakakibara et al. 2011).

Photodieldrin is the main metabolite from dieldrin, and a small amount of diol and other unidentified metabolites were also found in some cases. Dieldrin and trans-aldrin diol are the metabolic products of aldrin (Patil et al. 1972). Although no photolytic reaction could be observed in any of the control tubes which were illuminated in the absence of microorganisms, there is a possibility that a photosensitizing substance is present among the microbial products and the reaction is of photochemical-biochemical nature.

This conversion could be catalyzed by epoxide hydrolase, although there have been no studies focusing on the enzyme responsible for this conversion. Patil et al.

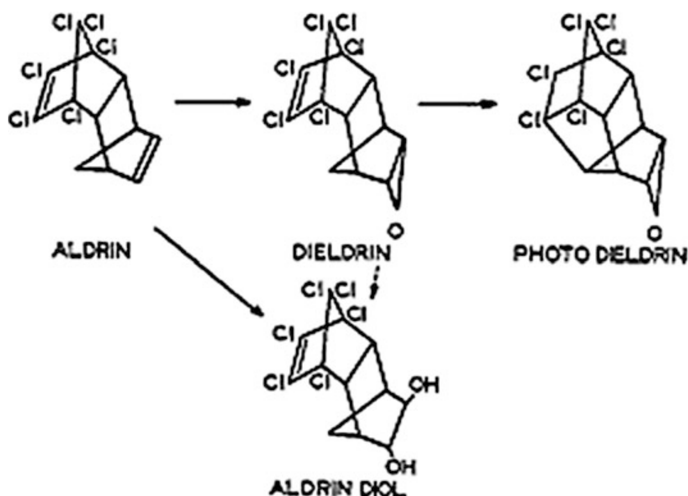


Fig. 2 Metabolite pathway of aldrin and dieldrin under oceanic conditions (Patil et al. 1972)

(1972) reported that in the metabolic transformation of dieldrin by marine microorganisms, photodieldrin was found as the major metabolic product of dieldrin (Fig. 2).

Several white rot fungi of *Phlebia* genus were able to degrade dieldrin. However, its metabolism differs from bacteria to bacteria. In this pathway, a large amount of 9-hydroxydieldrin was observed, suggest that the hydroxylation of dieldrin at the nine position is the major metabolic reaction, and the initial metabolic reaction of dieldrin seems to be similar to that in mammals. 9-hydroxydieldrin is further metabolized to dihydroxydieldrin, which hydroxylation reactions might play an important role in the metabolism of dieldrin, although the complete pathway of dieldrin is still unclear. The product monohydroxy 6,7-dihydroxydihydroaldrin could be produced from two alternate pathways: by hydroxylation of 6,7-dihydroxydihydroaldrin or by hydrolysis of monohydroxy-dieldrin at the epoxy ring. However, no trace of 6,7-dihydroxydihydroaldrin was observed in the present study. Thus, it seems that hydrolysis occurs at the epoxy ring of 9-hydroxydieldrin to produce a diol compound, which was probably 9-hydroxy-6,7-dihydroxydihydroaldrin. As no other monohydroxylated dieldrin was detected, only 9-hydroxydieldrin was found as main product of dieldrin from the cultures of three fungi (Fig. 3). Therefore, the methylene moiety of the dieldrin molecule seems to be the prior site for enzymatic attack in selected *Phlebia* species (Xiao et al. 2011).

It is generally known that aldrin is easily metabolized to dieldrin by multi-function oxidase of microbes or mammalians under aerobic conditions. It has been demonstrated that aldrin was initially branched to different metabolic routes, i.e. epoxidation, hydroxylation and oxidation, even though a large amounts of dieldrin were observed in selected fungal cultures with aldrin. This observation was

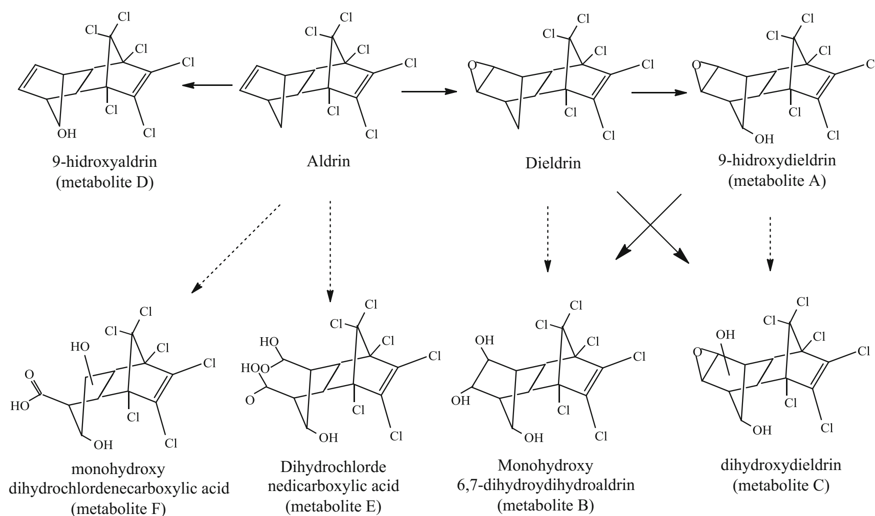


Fig. 3 Proposed pathway for the metabolism of aldrin and dieldrin by selected *Phlebia* species. The *broken arrows* indicate involvement of more than one step and the *dotted arrows* represent possible metabolic routes (Xiao et al. 2011)

supported by the result that 9-hydroxyaldrin and two carboxylic acid products were not detected from fungal cultures with dieldrin (Xiao et al. 2011).

The degradation pathway of aldrin and dieldrin has been shown in Fig. 3. A novel finding was the presence of 9-hydroxyaldrin, which was considered to be produced by hydroxylation at methylene of the aldrin molecule as dieldrin. The finding of 9-hydroxyaldrin further supports the hypothesis that the methylene bridge carbon of aldrin and dieldrin molecules is prone to enzymatic attack in selected WRF. Additionally, two carboxylated products were detected from these fungal cultures with aldrin. The dihydrochlordenedicarboxylic acid, a product resulting from oxidative ring cleavage of aldrin, has also been observed in some plants and soils (Klein et al. 1973; Kohli et al. 1973; Scheunert et al. 1977; Stewart and Gaul 1977), but it has not been detected in isolated bacteria and fungi. The formation of dihydrochlordenedicarboxylic acid suggests the ability of fungi to degrade aldrin by successive oxidation reactions.

However, Birolli et al. (2015) did not detect intermediate product that could be related to the degradation of dieldrin, suggesting that the dieldrin might be mineralized or transformed in polar xenobiotic compounds or CO_2 by conjugation reaction (Fig. 4). This is an important result for bioremediation purpose, since complete pesticide degradation or conjugation is desirable avoiding the presence of toxic or long-lasting products of biotransformation. The biotransformation, in the present of hydrogen peroxide, suggests the involvement of oxidoreductase in the pesticide degradation.

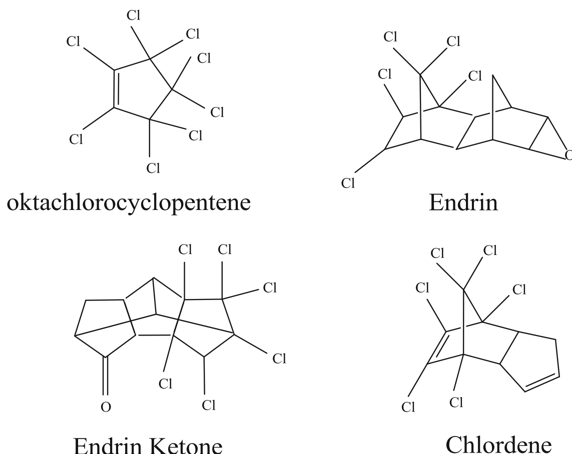


Fig. 4 Organochlorine compound identified by GC-MS in the sample of dieldrin biodegradation (Birolli et al. 2015)

5 Factors Effecting Degradation Process of Aldrin and Dieldrin

The efficiency of degrading microorganisms introduced into contaminated sites depends on many factors. In particular, the pollutant characteristics (e.g., concentration, bioavailability and microbial toxicity), the physicochemical characteristics of the environment, microbial ecology (e.g., predatory and competition), the characteristics of the degrading microorganisms and methodology for site remediation are dominant factors (Matsumoto et al. 2008, 2009). Therefore, it is important to understand the characteristics of the microorganisms and appropriate environmental conditions to achieve optimal degradation ability. Furthermore, it is necessary to isolate new competitive microorganisms that can degrade aldrin and dieldrin efficiently in natural environments as well as in the laboratory.

5.1 Effect of PH

The degradation of dieldrin was investigated at pH 4.0, 6.0, and 8.0 using soil fungus *Mucor racemous* strain DDF (Kataoka et al. 2010). About 90 % of dieldrin was degraded at all three pH values (Fig. 5). In the remediation study, *M. racemosus* strain DDF was found to degrade dieldrin under a wide range of pH. It is also advantageous that strain DDF was capable of growing rapidly in the soil compared to white rot fungi. Besides, Ferguson and Korte (1977) had reported that *Bacillus cereus*, *Bacillus subtilis* and *Nocardia* sp. degraded aldrin to exo-dieldrin, between pH range 5 and 9.5.

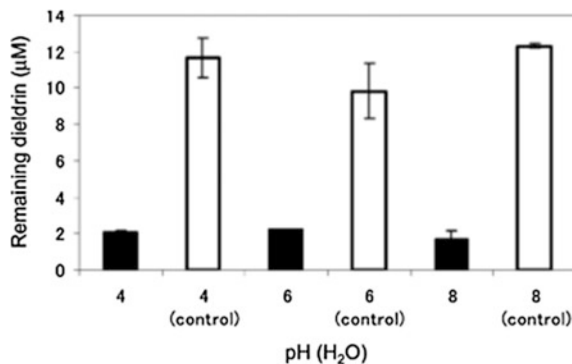


Fig. 5 Effect of pH of the medium on dieldrin degradation by *M. racemous* strain DDF

5.2 *Effect of Carbon and Nitrogen Concentrations*

The maximum degradation of dieldrin by *M. racemous* strain DDF was achieved up to 89.8 % at glucose concentration of 1.0 % (Kataoka et al. 2010). However, there was significant difference in degradation of dieldrin after addition of glucose concentrations of 0.5, 1.0, and 2.0 %. In contrast, lower dieldrin degradation was observed at addition of glucose concentrations of 0.1 and 10 % (Fig. 6).

Dieldrin was degraded over the range of added nitrogen concentrations, as reflected in Fig. 7. However, there were no significant differences among 0.5-, 1-, and 2-folds variations in nitrogen concentrations.

The results clearly indicated that *M. racemous* strain DDF degraded dieldrin under a wide range of carbon and nitrogen source concentrations. At low concentrations of nitrogen or carbon sources, a low degradation capability of strain DDF was observed (Figs. 6, 7). The hyphal growth was poor at the low concentrations of glucose and nitrogen, probably due to nutrient deficiency, and at 10 % glucose due to high osmotic pressure (data not shown). These results indicate that strain DDF needs a suitable growth situation to degrade dieldrin (Kataoka et al. 2010).

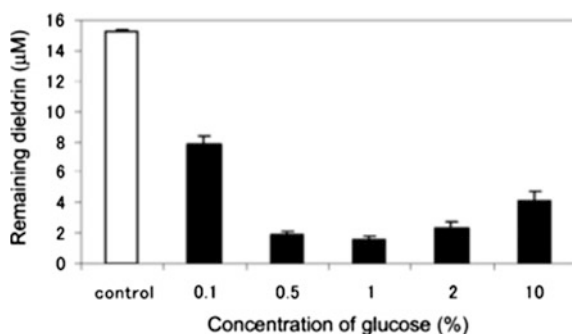


Fig. 6 Effect of carbon in the medium on dieldrin degradation by *M. racemous* strain DDF

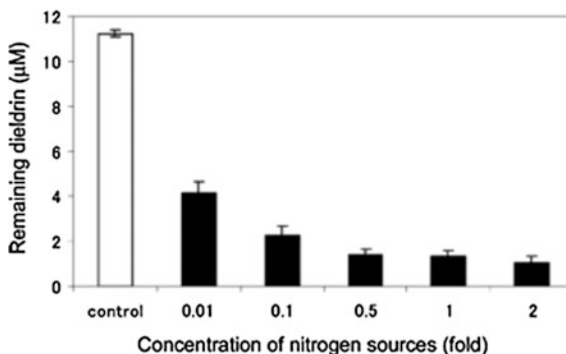


Fig. 7 Effect of nitrogen in the medium on dieldrin degradation by *M. racemous* strain DDF

Huggenholtz and MacRae (1990) investigated the effect of carbon amendments and analogues on the disappearance of aldrin and dieldrin residues from the soil. Soil samples, supplemented with xylose and inositol, showed a marked decrease in detectable concentrations of aldrin (84–96 %) and dieldrin (50–79 %), after 60 days, relative to untreated and zero time controls. Decalin, borneol and isoborneol treated soils produced a statistically significant decrease in aldrin concentration (27–74 %) in the same period of time relative to zero time control and untreated soil control. The remaining amendments had no appreciable effect on the levels of insecticide residues in the soil (Table 2). Biodegradation of aldrin and dieldrin in the test soil appears to be stimulated by the addition of xylose and inositol. These amendments may be acting as growth substrates for the co-metabolism of aldrin and dieldrin by the soil microflora. Growth substrates perhaps, provide co-factors or metabolites necessary for the catalytic activity of enzymes (usually non-specific) capable of transforming xenobiotics (Janke and Fritsche 1985).

5.3 Effect of the Extracellular Fluid (Involvement of Epoxide Hydrolase Enzyme)

Kataoka et al. (2010) used the extracellular fluid of the spent broth culture for the dieldrin degradation experiments. First, the extracellular fluid was concentrated by ultrafiltration, and the ultrafiltrate was further fractionated with ammonium sulfate. The ultra filtrate degraded dieldrin by 46 % compared to control. Besides, degradation of dieldrin was observed in the fraction corresponding to 55–75 % saturation of ammonium sulfate, whereas almost no degradation was observed in the fraction corresponding to 35–45 % saturation. The highest degradation was observed in the 55 % fraction (Fig. 8). *Aspergillus niger* is known to produce epoxide hydrolase (Faber et al. 1996). This enzyme involves in the reaction and causes opening of the epoxide, leading to the formation of the trans-diol. Thus, it is evident that epoxide hydrolase was involved in the transformation of dieldrin to aldrin trans-diol.

Table 2 Effect of selected amendments on the detectable aldrin and dieldrin in soil over a 60 days incubation period at 28 °C (Huggenholtz and MacRae 1990)

Amendment	Incubation time (days)	Average concentration in soil (ng g^{-1})	
		Aldrin	Dieldrin
Non	0 15 60	7025 ± 399 7348 ± 818 7578 ± 2198	3763 ± 362 3662 ± 489 3385 ± 1677
Amino acids	0 15 60	6495 ± 351 7944 ± 1754 7490 ± 3303	3638 ± 540 3140 ± 588 4236 ± 2836
Glucose	0 15 60	5735 ± 723 6789 ± 1391 4255 ± 4088	3210 ± 66 4088 ± 1490 2453 ± 1235
Inositol	0 15 60	6954 ± 2448 6085 ± 1846 548 ± 175	3530 ± 518 2952 ± 880 1156 ± 204
Xylose	0 15 60	8221 ± 657 7684 ± 5085 535 ± 155	4112 ± 305 3378 ± 1916 1296 ± 386
CHC	0 15 60	7659 ± 138 5624 ± 809 7041 ± 1360	5212 ± 1252 3239 ± 448 4571 ± 1260
DCCH	0 15 60	7436 ± 177 8852 ± 2670 6314 ± 1796	4834 ± 1786 6420 ± 3159 3636 ± 821
Decalin	0 15 60	7338 ± 37 6127 ± 962 3781 ± 1367	4046 ± 671 2417 ± 576 2918 ± 628
Borneol	0 15 60	7485 ± 171 7103 ± 2875 3673 ± 1661	4250 ± 382 3680 ± 1110 633 ± 1289
Isoborneol	0 15 60	7393 ± 301 7450 ± 2240 3630 ± 833	4007 ± 38 4040 ± 1860 2757 ± 849

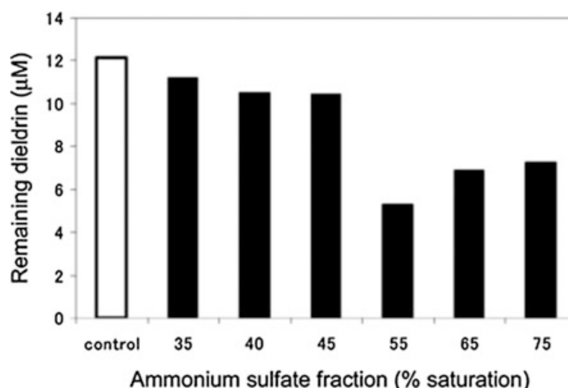


Fig. 8 Degradation of dieldrin by the extracellular fluid from *M. racemous* strain DDF

5.4 Effects of Variations in Light on Growth and Conversion

Brain and Lines (1982) used *Phaseolus vulgaris* cell culture to degrade aldrin and dieldrin. Effect of variations in light was investigated in cultures fed with 10 mg [^{14}C] aldrin or dieldrin, keeping under light or dark conditions throughout the culture period (Table 3). In case of aldrin, both treatments resulted in the production of dieldrin, aldrin-trans-dihydrodiol and polar material, but no photodieldrin and polar material were produced. However, uptake and conversion of aldrin was slow in the dark-grown cultures. It was noted that the dieldrin-treated cultures, grown in the dark, appeared considerably darker than those treated with aldrin, or left untreated, and kept in the dark. Photodieldrin was only inside the cells. These results confirmed that production of photodieldrin took place only after uptake and its production was independent of the presence of light. Thus photodieldrin is a true metabolite of dieldrin in the plant system.

5.5 Effect of Variation in Hormonal Levels

10 mg [^{14}C] aldrin and [^{14}C] dieldrin were added to cultures in seven closely related well-defined media based on a commercial formulation and supplemented

Table 3 Uptake and metabolism of [^{14}C] aldrin and dieldrin by suspension cultures from *Phaseolus vulgaris* root under light and dark conditions

		Light (%)	Dark (%)
Aldrin added			
Growth inhibition		10	10
Aldrin	Medium Cells total	34 11 45	37 10 47
Dieldrin	Medium Cells total	11 26 37	9 31 40
Aldrin-trans-diol		4	4
Polar metabolites		3	4
Total conversion		43	48
Dieldrin added			
Growth inhibition		42	46
Dieldrin	Medium Cells total	46 36 82	75 11 87
Photodieldrin		6	4
Polar metabolites		6	3
Total conversion		12	7

with varying quantities and ratios of 2,4-D and kinetin in the range of 0.5–2 mg L^{-1}. Cells were grown on a medium containing 0.5 mg L^{-1} 2,4-D and transferred to media containing higher levels of these hormones and conditioned for 8 weeks before addition of substrates (Brain and Lines 1982).

Both aldrin and dieldrin were taken up and converted to the usual products, although there were quantitative differences in the rates of microbial growth, uptake and metabolism. In case of aldrin, there was some correlation between uptake into the cells and accumulation of dieldrin. With regard to conversion, there was a significant variation from medium to medium in the distribution between dieldrin, aldrin-trans-dihydro-diol and polar material. In case of dieldrin, there was a similar correlation between uptake and conversion to photodieldrin and polar metabolites, but no correlation between concentration and growth inhibition. The highest rates of uptake and conversion were found in the standard medium.

Thus, it may be concluded that a variations in hormonal supplementation had a impact on the uptake and metabolism of aldrin and dieldrin. The maximum extent of variation was less than four folds. Minor variation in medium composition can therefore be excluded as an important cause for variability in response of cultures. However, it is still possible that change of hormone can produce significant effects qualitatively.

6 Pesticide Toxicity to Microbes

Pesticides are extensively used in agriculture as a part of pest control strategies. Owing to their xenobiotic characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated biotransformation in the soil. Many studies show that pesticides reduce activities of soil enzymes that are key indicators of soil health. The applied pesticides may also influence many biochemical reactions, such as mineralization of organic matter, nitrification, denitrification, ammonification, redox reactions, methanogenesis, etc. The amount of applied pesticides reaching the target organism is about 0.1 %, while the remaining contaminates the soil environment (Carriger et al. 2006; Pimentel 1995). With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and microbial processes has received more attention (Andrea et al. 2000; Baxter and Cummings 2008). The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering in the food chain. Adverse impacts of pesticides on soil microbial diversity and activities have been described by Ingram et al. (2005).

Pesticides also influence soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated biotransformations, such as nutrient dynamics and their bioavailability, are also more or less adversely affected by the pesticides (Demanou et al. 2004).

The applied pesticides also reduce soil enzymatic activities that act as a "biological index" of soil fertility and other biological processes in the soil environment (Antonious 2003).

Pesticides also have an impact on soil microbial biomass and soil respiration. Generally, a decrease in soil respiration reflects the reduction in microbial biomass (Chen and Edwards 2001) or increase in respiration implies the enhanced growth of bacterial population (Wardle et al. 1994). However, some microbial groups are capable of using applied pesticide as a source of energy and nutrients to multiply. Sometimes, application of pesticides reduces microbial diversity, but increases functional diversity of microbial communities (Wang et al. 2006) and also demonstrate the tendency of reversible stimulatory/inhibitory effects on soil microorganisms. Pesticides application may inhibit or kill certain group of microorganisms and out number other groups by making them out of competition. Chen and Edwards (2001) reported that fungicides application killed or inhibited the activity of certain fungi which led to a rapid flush of bacterial activity. In the other research, Lopez et al. (2006) reported that heterotrophic mesophilic and psychrophilic aquatic bacteria as well as culturable phosphate-solubilizing microorganisms increased in lake water when treated with herbicide simazine.

6.1 Factors Regulating Pesticide Toxicity in Microbes

The effect of a pesticide on soil microorganisms is controlled by numerous environmental factors as well as persistence, concentration, toxicity of the applied pesticide, and its bioavailability (Abdel-Mallek et al. 1994). One of the major factors contributing to the net impact of applied pesticides on soil microbes is its bioavailability in soil environment. Adsorption and desorption processes regulate concentration of a contaminant in soil solution (Takagi 2008) and hence its bioavailability, bioactivity, and degradability in soil environment.

In addition to soil texture, presence of organic matter and vegetation also influences pesticide toxicity to the microbes in the soil environment. Addition of carbon sources including glucose, acetate, and some amino acids (glutamine, arginine, serine, and tryptophan) fungal enhances resistance to pesticide toxicity (Mishra and Pandey 1989).

Under natural soil conditions, there may be many pesticides together or other contaminants in addition to pesticides at a time in a single locality. The coexistence of different pesticides and other contaminants might have different impacts on microbial activity and diversity. Wang et al. (2006) evaluated the effect of methamidophos and urea on microbial diversity in soil by using integrated approaches of soil microbial biomass analysis and community level physiological profiles (CLPPs). They concluded that agrochemicals reduced microbial biomass and enhanced functional diversities of soil microbial communities; i.e., some species of bacteria might be enriched in soils under methamidophos stress (Wang et al. 2006).

Saez et al. (2006) observed the effect of some pesticides (aldrin, lindane, dimethoate, methyl parathion, methidathion, atrazine, simazine, captan, and diflubenzuron) on growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064. The herbicide atrazine and an insecticide dimethoate completely inhibited growth and biological activity of *X. autotrophicus* at 10 mg L^{-1}, while the rest of the tested pesticides delayed the growth of strain *X. autotrophicus* CECT 7064, but did not drastically affect the bacterial growth after 96 h of culture. The denitrifying activity of *X. autotrophicus* was negatively affected by the pesticides application with the exception of fungicide captan. The release of N_2O was strongly inhibited by several pesticides (aldrin, lindane, methyl parathion methidathion, and diflubenzuron), while dimethoate, atrazine, and simazine inhibited totally the denitrifying activity of the bacteria (Saez et al. 2006).

Virag et al. (2007) studied the effects of pesticides and their degradative products, produced by UV treatment, on microbiological activity. They selected five photosensitive pesticides (carbendazim, acetochlor, simazine, EPTC, and chlorpyrifos) and six representative soil microbes (*Bacillus subtilis, Pseudomonas fluorescens, Mycobacterium phlei, Fusarium oxysporum, Penicillium expansum,* and *Trichoderma harzianum*) for their experiments. Among them, acetochlor and its degradation products were more toxic to bacteria than fungi. All bacterial strains were sensitive to the parent compound and its degradation products as well. End product of zarbendazim was moderately toxic against *P. fluorescens* and *B. subtilis*, but strongly toxic to *T. harzianum*. Chlorpyrifos and its metabolites did not inhibit the test organisms. They concluded that the pesticide photodegradation could result in significant changes in soil microbiota in addition to the formation of biologically harmful degradation products.

6.2 *Toxicity of Organochlorine Pesticides*

Organochlorines are the most successful, profitably utilized and commercialized group of pesticides. They have gained huge popularity and prominence in a short span of time by virtue of their ability to control almost all kinds of pests including insect, fungi, rodent, etc. The toxicity of an individual pesticide to the pests is predominantly determined by its structure, the different moieties attached to parent compound, their spatial arrangements within molecule, nature of substituents, polarity, symmetry and asymmetry of molecules, the solubility and sorption values.

Aldrin is derived from hexachlorocyclopentadiene. Insecticidal properties of aldrin were first reported in 1945. The active ingredient is highly insecticidal, but has a relatively short residual life under field conditions at normally applied concentrations. Isodrin, which is a stereoisomer of aldrin, is known to possess much more toxicity against many insects at equivalent concentrations of aldrin. Another analogue from the hexachlorocyclopentadiene family, named dieldrin, is also insecticidal (Kearns et al. 1945). It is formed as a result of epoxidation of aldrin. It

is highly effective against mosquito larvae, flies, ants, fleas, ticks, lice, earwigs and other household pests and is also one of the longest residual active chemical.

Both endrin and isodrin are stereoisomers of aldrin. All these compounds share a common property, i.e. presence of hexachlorocyclopentadiene group, which is the primary chemical moiety that shows toxicidal activities. Further, different substituents either decrease or increase the toxicity. The presence of double bond in the ring increases the toxicity, whereas its epoxidated product dieldrin shows decreased toxicity which was not very significant. Toxicity is also directly related to endo–endo, endo–exo attachment of the rings. In both aldrin and dieldrin, the attachments are mainly exo–endo. In endrin, this attachment is exo–exo, therefore the oral LD50 to rats is very low (7.5–17.5 mg kg^{-1}) in comparison to that of aldrin (39 mg kg^{-1}) and dieldrin (46 mg kg^{-1}) (Fig. 9, Treon et al. 1955).

The effect of a pesticide on soil microorganisms is controlled by numerous environmental factors in addition to the persistence, concentration, toxicity of the applied pesticide and its bioavailability. The mode of action of the pesticide in target organism is closely associated with the structure of pesticidal compound. The parent molecule of compound is not only responsible for the activity, but also the nature of substituents, presence of the epoxide ring, double–triple bond, conjugation, aromaticity and the stereochemistry determine the toxicity of the pesticidal compound. So understanding of the structure of compounds and their correlation

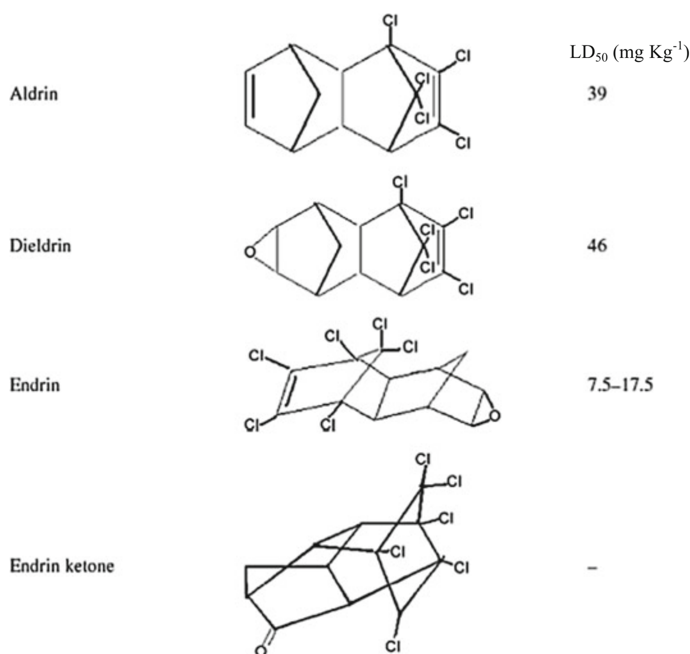


Fig. 9 Toxicity (LD_{50}) of aldrin and other analog compounds

with toxicity to target organism is a very important parameter for developing better designed pesticidal compounds with tailored toxicidal properties on different pests.

7 Future Prospective

The use of microorganisms for bioremediation requires an understanding of all the biochemical aspects of pesticides transformation. Many studies had reported degradation of aldrin, and dieldrin—a main metabolite of aldrin is more toxic and persistent than aldrin. Even though some microorganisms can further transform dieldrin, degradation rate is very low and hence needs long time for treatment. Identification of metabolic products of dieldrin is still limited and 9-hydroxyldieldrin is the most common one. Optimization of the experimental conditions is required to improve the degradation rates and also to identify other metabolic products form dieldrin, which are less toxic and simple. The finding of new microorganisms, which can degrade aldrin and dieldrin completely and fast, is still underway. Mechanism of degradation aldrin and dieldrin especially through involvement of enzymes has to be explored to identify the genes encoding these enzymes. Thus, there is a need for the development of molecular strategies to treat organochlorine-polluted soil systems.

8 Conclusion

Aldrin and dieldrin are extensively used as synthetic pesticides in the agricultural industry. Hence, it has become a serious environmental problem and these pesticides are classified as persistent organic pollutants (POPs). Although these compounds have been prohibited over the past decades in most countries, they are still found in the environment, especially in the soils of agricultural fields. Biodegradation, using bacteria and fungi, has been found to be a cost-effective and eco-friendly method for treating various pesticides including aldrin and dieldrin, which have been in use since the late 1960s.

Many studies have shown the microbial transformation of aldrin and dieldrin to intermediate metabolites, although the metabolic pathways are still unclear. In general, degradation of aldrin and dieldrin by fungi is faster than that of bacteria of which degradation pathway is clearly described. The efficiency of degrading microorganisms, introduced into contaminated sites, depends on many factors such as, pH, carbon and nitrogen sources, enzymes, hormones, and light. Some enzymes had been found to be involved in aldrin and dieldrin degradation, such as glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, β-glucorinadase, epoxide hydrolase, etc. Most of studies reported that dieldrin is main metabolic product of aldrin, which has higher toxicity and persistence. However, some microorganisms had ability to convert dieldrin to some

metabolic compounds such as aldrin dicarboxyric acid, photodieldrin, aldrin diol, 9-hydroxydieldrin, dihydroxydieldrin, endrin, chlordane, etc. However, some studies have also revealed that dieldrin could be mineralized to CO_2.

Acknowledgments This work was supported by a grant for the Research Project supported by International Research Collaboration and Scientific Publication 2016, from the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia.

References

Abdel-Mallek, A. Y., Moharram, A. M., Abdel-Kader, M. I., & Omar, S. A. (1994). Effect of soil treatment with the organophosphorus insecticide profenofos on the fungal flora and some microbial activities. *Microbiological Research, 149*, 167–171.

Andrea, M. M., Peres, T. B., Luchini, L. C., & Pettinelli, A, Jr. (2000). Impact of longterm pesticide application on some soil biological parameters. *Journal of Environmental Science and Health Part B, 35*, 297–307.

Antonious, G. F. (2003). Impact of soil management and two botanical insecticides on urease and invertase activity. *Journal of Environmental Science and Health Part B, 38*, 479–488.

Bandala, E. R., Paulino, P., Juan, A. O., & Luis, G. T. (2006). Removal of aldrin, dieldrin, heptachlor, and heptachlor epoxide using activated carbon and/or *Pseudomonas fluorescens* free cell cultures. *Journal of Environmental Science and Health Part B, 41*, 553–569.

Baxter, J., & Cummings, S. P. (2008). The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil. *Journal of Applied Microbiology, 104*, 1605–1616.

Birolli, G. W., Yamamoto, K. Y., Oliveira, J. R., Nitschke, M., Seleghim, M. H. R., & Porto, L. M. (2015). Biotransformation of dieldrin by the marine fungus *Penicillium miczynskii CBMAI 930*. *Biocatalysis and Agricultural Biotechnology, 4*, 39–43.

Brain, K. R., & Lines, D. S. (1982). Consistency of metabolism of aldrin and dieldrin in suspension cultures from *Phaseolus vulgaris* root. *Plant Cell Reports, 1*, 176–179.

Carriger, J. F., Rand, G. M., Gardinali, P. R., Perry, W. B., Tompkins, M. S., & Fernandez, A. M. (2006). Pesticides of potential ecological concern in sediment from South Florida canals: An ecological risk prioritization for aquatic arthropods. *Soil and Sediment Contamination, 15*, 21–45.

Chen, S. K., & Edwards, C. A. (2001). A microcosm approach to assess the effects of fungicides on soil ecological processes and plant growth: Comparison of two soil types. *Soil Biology and Biochemistry, 33*, 1981–1991.

Demanou, J., Monkiedje, A., Njine, T., Foto, S. M., Nola, M., Serges, H., et al. (2004). Changes in soil chemical properties and microbial activities in response to the fungicide Ridomil gold plus copper. *International Journal of Environmental Research and Public Health, 1*, 26–34.

Faber, K., Mischitz, M., & Kroutil, W. (1996). Microbial epoxide hydrolases. *Acta Chemica Scandinavica, 50*, 249–258.

Ferguson, J. A., & Korte, F. (1977). Epoxidation of aldrin to exo-dieldrin by soil bacteria. *Applied and Environmental Microbiology, 34*, 7–13.

Foght, J., April, T., Biggar, K., & Aislabie, J. (2001). Bioremediation of DDT-contaminated soils: A review. *Bioremediation Journal, 5*, 225–246.

Huggenholtz, P., & MacRae, I. C. (1990). Stimulation of aldrin and dieldrin loss from soils treated with carbon amendments and saturated-ring analogues. *Bulletin of Environmental Contamination and Toxicology, 45*, 223–227.

Ingram, C. W., Coyne, M. S., & Williams, D. W. (2005). Effects of commercial diazinon and imidacloprid on microbial urease activity in soil. *Journal of Environmental Quality, 34*, 1573–1580.

Janke, D., & Fritsche, W. J. (1985). Nature and significance of microbial cometabolism of xenobiotics. *Basic Microbiology, 25*, 603–619.

Jensen, G. E., Skaare, J. U., & Egaas, E. (1991). Response of xenobiotic metabolizing enzymes in rainbouw trout (*Onchohypus mykiss*) to endosulfan, detected by enzyme activities and immunochemical methods. *Aquatic Toxicology, 21*, 81–92.

Kamei, I., Takagi, K., & Kondo, R. (2010). Bioconversion of dieldrin by wood rotting fungi and metabolite detection. *Pest Management Science, 66*, 888–891.

Kataoka, R., Takagi, K., Kamei, I., Kiyota, H., & Sato, Y. (2010). Bidegradation of dieldrin by a soil fungus isolated from a soil with annual endosulfan applications. *Environmental Science and Technology, 44*, 6343–6349.

Kearns, C. W., Ingle, L., & Metcalf, R. F. (1945). New chlorinated-hydrocarbon insecticide. *Journal of Economic Entomology, 38*, 661–668.

Klein, W., Kohli, J., Weisgerber, I., & Korte, F. (1973). Fate of aldrin-^{14}C in potatoes and soil under outdoor conditions. *Journal of Agricultural and Food Chemistry, 21*, 152–156.

Kohli, J., Zarif, S., Weisgerber, I., Klein, W., & Korte, F. (1973). Fate of aldrin-^{14}C in sugar beets and soil under outdoor conditions. *Journal of Agricultural and Food Chemistry, 21*, 855–857.

Lichtenstein, E. P., & Schulz, K. R. (1960). Epoxidation of aldrin and heptachlor in soils as influenced by auto claving, moisture and soil types. *Journal of Economic Entomology, 53*, 192–197.

Lichtenstein, E. P., Schulz, K. R., & Cowley, G. T. (1963). Inhibition of the conversion of aldrin to dieldrin in soils with methylenedioxyphenyl synergists. *Journal of Economic Entomology, 56*, 485–489.

Lichtenstein, E. P., Schulz, K. R., Skrentny, R. F., & Stitt, P. A. (1965). Insecticidal residues in cucumbers and alfalfa grown on aldrin- or heptachlortreated soils. *Journal of Economic Entomology, 58*, 742–746.

Lopez, L., Pozo, C., Rodelas, B., Calvo, C., & Gonzalez-Lopez, J. (2006). Influence of pesticides and herbicides presence on phosphatase activity and selected bacterial microbiota of a natural lake system. *Ecotoxicology, 15*, 487–493.

Luckens, M. M., & Phelps, K. I. (1969). *Serum enzyme patterns in acute poisoning with organochlorine insecticides* (Vol. 17, p. 4). Madison: Department of Entomology, University of Winconsin.

Matsumoto, E., Kawanaka, Y., Yun, S. J., & Oyaizu, H. (2008). Isolation of dieldrin- and endrin-degrading bacteria using 1,2-epoxycyclohexane as a structural analog of both compounds. *Applied Microbiology and Biotechnology, 80*, 1095–1103.

Matsumoto, E., Kawanaka, Y., Yun, S. J., & Oyaizu, H. (2009). Bioremediation of the organochlorine pesticides, dieldrin and endrin and their occurrence in the environment. *Applied Microbiology and Biotechnology, 84*, 205–216.

Matsumura, F., & Boush, G. M. (1967). Dieldrin: Degradation by soil microorganisms. *Science, 156*, 959–961.

Matsumura, F., & Boush, G. M. (1968). Degradation of insecticides by a soil fungus, *Trichoderma viride*. *Journal of Economic Entomology, 61*, 610–612.

Matsumura, F., Patil, K. C., & Boush, G. M. (1970). Formation of "photodieldrin" by microorganisms. *Science, 170*, 1206–1207.

Matthews, H. B., & Matsumura, F. (1969). Metabolic fate of dieldrin in the rat. *Pesticide Biochemistry and Physiology, 14*, 192–207.

Maule, A., Plyte, S., & Quirk, A. V. (1987). Dehalogenation of organochlorine insecticides by mixed anaerobic microbial populations. *Pesticide Biochemistry and Physiology, 27*, 229–236.

Mishra, A. K., & Pandey, A. B. (1989). Toxicity of three pesticides to some nitrogen fixing cyanobacteria. *Ecotoxicology and Environmental Safety, 17*, 236–246.

Mullin, C. A., & Wilkinson, C. F. (1980). *Insect epoxide hydrolase: Properties of a purified enzyme from the southern armyworm (Spodoptera eridania)*. New York: Department of Entomology, University of Cornell.

Osburn, W. O., & Kensler, T. W. (2008). Nrf2 signaling: An adaptive response pathway for protection against environmental toxic insults. *Mutation Research, 659*, 31–39.

Patil, K. C., Matsumura, F., & Boush, G. M. (1970). Degradation of endrin, aldrin, and DDT by soil microorganisms. *Applied Microbiology, 19*, 879–881.

Patil, K. C., Matsumura, F., & Boush, G. M. (1972). Metabolic transformation of DDT, dieldrin, aldrin and endrin by marine microorganisms. *Environmental Science and Technology, 6*, 629–632.

Pimentel, D. (1995). Amounts of pesticides reaching target pests: Environmental impacts and ethics. *Journal of Agricultural and Environmental Ethics, 8*, 17–29.

Reddy, M. S., & Jayaprada, P. (1991). Toxic impact of aldrin on acid and alkaline phospatase activity of penaeid prwn, *Metapenaeus monoceros* in vitro study. *Bulletin of Environmental Contamination and Toxicology, 46*, 479–484.

Saez, F., Pozo, C., Gomez, M. A., Martınez-Toledo, M. V., Rodelas, B., & Gonzalez- Lopez, J. (2006). Growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064 in the presence of selected pesticides. *Applied Microbiology and Biotechnology, 71*, 563–567.

Sakakibara, F., Kazuhiro, T., Ryota, K., Hiromasa, K., Yuuki, S., & Sanae, O. (2011). Isolation and identification of dieldrin-degrading *Pseudonocardia* sp. Strain KSF27 using a soil–charcoal perfusion method with aldrin trans-diol as a structural analog of dieldrin. *Biochemical and Biophysical Research Communications, 411*, 76–81.

Scheunert, I., Kohli, J., Kaul, R., & Klein, W. (1977). Fate of [^{14}C] aldrin in crop rotation under outdoor conditions. *Ecotoxicology and Environmental Safety, 1*, 365–385.

Schonbrod, R. D., Hoyers, S. J., & Yu, S. J. (1973). The epoxidation of aldrin by microsomes from the Fc strain of housefly: A genetic survey. In *Pesticide Biochemistry and Physiology* (pp. 259–270). Department of Entomology, Oregon State University.

Stewart, D. K. R., & Gaul, S. O. (1977). Dihydrochlordene dicarboxylic acid residues in soil treated with high rates of aldrin. *Bulletin of Environmental Contamination and Toxicology, 17*, 712–713.

Takagi, T. (2008). Surfactant effects on environmental behavior of pesticides. *Reviews of Environmental Contamination and Toxicology, 194*, 1–177.

Treon, J. F., Clevelend, F. P., & Cappel, J. (1955). Toxicity of endrin for laboratory animals. *Journal of Agricultural and Food Chemistry, 3*, 842–848.

Tu, C. M., Miles, J. R. W., & Harris, C. R. (1968). Soil microbial degradation of aldrin. *Life Sciences, 7*, 311–322.

Virag, D., Naar, Z., & Kiss, A. (2007). Microbial toxicity of pesticide derivatives roduced with UV-photodegradation. *Bulletin of Environmental Contamination and Toxicology, 79*, 356–359.

Walker, C. H. (1978). Spesies differences in microsomal monooygenase activity and their relationship to biological half-lives. *Drug Metabolism Reviews, 7*, 295–323.

Wang, M. C., Gong, M., Zang, H. B., Hua, X. M., Yao, J., Pang, Y. J., et al. (2006). Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. *Journal of Environmental Science and Health Part B, 41*, 399–413.

Wardle, D. A., Nicholson, K. S., & Rahman, A. (1994). Influence of herbicide applications on the decomposition, microbial biomass, and microbial activity of pasture shoot and root litter. *New Zealand Journal of Agricultural Research, 37*, 29–39.

World Health Organization. (1989). *Environmental health criteria 91, Aldrin and Dieldrin*. Geneva: World Health Organization.

Xiao, P., Mori, T., Kamei, I., Kiyota, H., Takagi, K., & Kondo, R. (2011). Novel metabolic pathway of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus *Phlebia*. *Chemosphere, 85*, 218–224.

Yamazaki, K., Takagi, K., Kataoka, R., Kotake, M., Yamada, T., & Kiyota, H. (2014). Novel phosphorylation of aldrin-trans-diol by dieldrin-degrading fungus Mucor racemosus strain DDF. *International Biodeterioration and Biodegradation, 92*, 36–40.

Yu, S. J., & Terriere, L. C. (1972). Enzyme induction in the housefly: The specificity of the cyclodiene insecticides. In *Pesticide biochemistry and physiology* (Vol. 2, pp. 184–190). Corvallis, Oregon: Department of Entomology, Oregon State University.

Microbial Degradation of Chlorophenols

Muftah H. El-Naas, Hussein A. Mousa and Maisa El Gamal

1 Introduction

Chlorophenols (CPs) are common environmental pollutants that are usually introduced into the environment as a result of chemical and pharmaceutical industrial activities (Jensen 1996; Czaplicka 2004; Michałowicz and Duda 2007). The prevalence of these pollutants in the environment is associated with the production, use and degradation of numerous pesticides which can easily find their ways into the environment (Hale et al. 1994). The main potential environmental sources of chlorophenols include: (a) direct soil application as biocides; (b) synthesis during engineering processes where chlorine is used; (c) leaching and vaporizing from treated wood items; (d) release or discharge from factories into air and water; and (e) combustion of organic material in the presence of chloride.

Chlorophenols have become today important pollutants in the environment and their treatment, disposal, and general management have become serious challenges to environmental agencies in most parts of the world (Weber et al. 2008). The diversity, versatility, adaptability, and metabolic potentials of a number of microbes have been harnessed and applied in the bioremediation of numerous environmental contaminants. In an effort to remediate the effects of chlorophenols, bioremediation using microorganisms has been suggested by many workers (Pandey et al. 2003; Czaplicka 2004). However, a number of contaminants have been shown to be refractory to microbial degradation; hence they are either not metabolizable or are

M.H. El-Naas (✉)
Gas Processing Center, College of Engineering,
Qatar University, P.O. Box 2713, Doha, Qatar
e-mail: muftah@qu.edu.qa

H.A. Mousa · M.E. Gamal
Chemical and Petroleum Engineering Department,
UAE University, P.O. Box 15551, Al-Ain, United Arab Emirates

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_2

transformed into other metabolites that accumulate in the environment (Esteve-Núñez et al. 2001).

Phenol and its derivatives, such as chlorophenols are among the most common environmental pollutants that are often used as agricultural pesticides. They are also produced during the preparation of paper and the burning of organic materials in the presence of chloride (Antizar-Ladislao and Galil 2004). Microorganisms, such as bacteria, fungi, actinomycete, protozoa, etc. have been found to be effective in the degradation and recycling of organic pollutants. Microorganisms, that are capable of degrading organic contaminants, can be isolated, cultured, adapted, and enriched under laboratory conditions. Indigenous microbial populations, especially heterotrophic bacteria and fungi, can play a key role in the biodegradation process of toxic and recalcitrant pollutants.

It has been reported that a fraction of the soil biota containing microorganisms, used to bio convert chlorophenols rapidly and also develop the ability to degrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors to certain soil microorganisms which are required for the bioconversion of pesticide-contaminated sites (Qiu et al. 2007; Araya and Lakhi 2004). It was also observed that microorganisms, that have the ability to degrade pesticides, can also bio-remediate other chemical compounds as well (Singh and Walker 2006). The transformation of such contaminants depends not only on the presence of microorganisms with appropriate degrading enzymes, but also on a wide range of environmental parameters (Aislabie and Lloyd-Jones 1995). Moreover, the ecological, biochemical, molecular and physiological factors play a significant role in the biodegradation process of such pollutants (Iranzo et al. 2001; Vischetti et al. 2002).

This chapter addresses the state of chlorophenolic compounds in the environment with special emphasis on their microbe-induced degradation. The biodegradation of CPs has been reviewed with respect to CPs degrading microbes, factors for degradability and degradation mechanisms. The enzymes involved in the microbial degradation of CPs have also been discussed. However, this chapter mostly focuses on the degradation mechanism of 2,4-dichlorophenol and pentachlorophenol.

2 Structures of Chlorophenols

Chlorophenols (CPs) are a group of chemicals in which chlorines are attached between C1 and C5 of phenol, which is an aromatic compound derived from benzene by adding a hydroxyl group to a carbon to replace hydrogen. There are five basic types of CPs: monochlorophenols, dichlorophenols, trichlorophenols, tetrachlorophenols, and pentachlorophenols. In all, there are 19 different CPs which have been shown in Fig. 1 (Van Agteren et al. 1998).

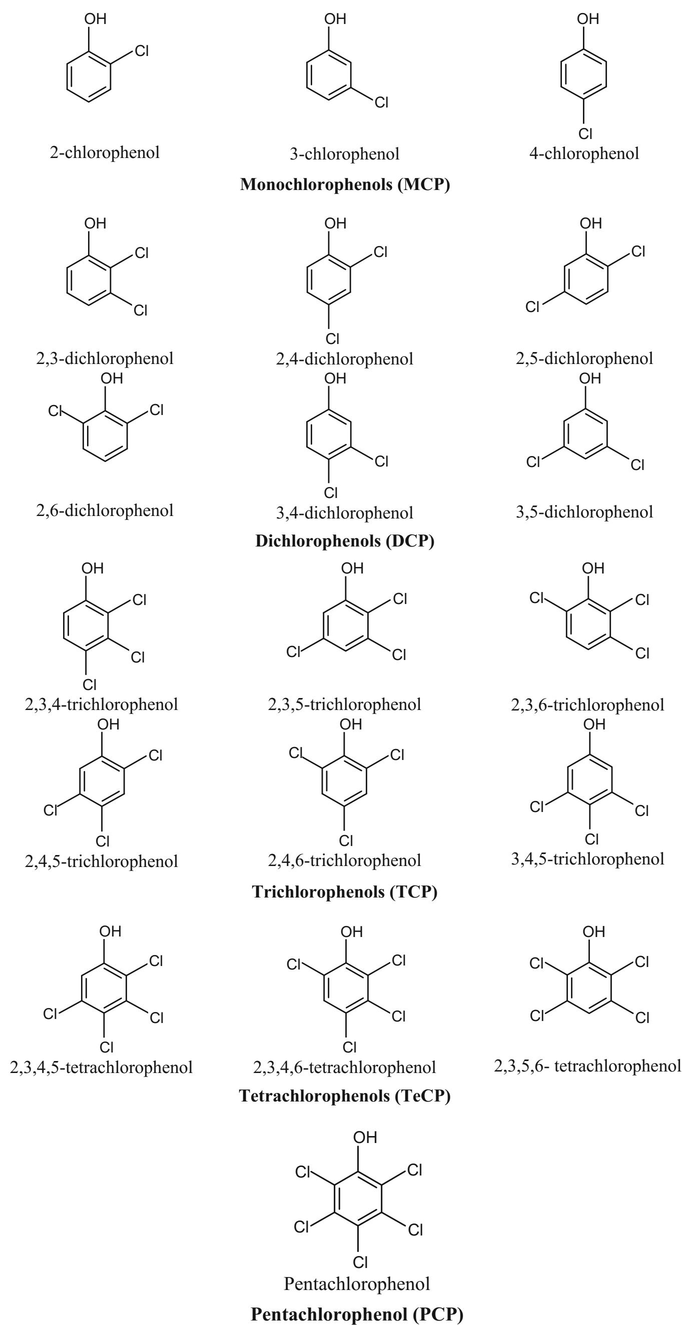


Fig. 1 Chemical structure of chlorophenols (Van Agteren et al. 1998)

3 Physical and Chemical Properties of Chlorophenols

The CPs consists of a benzene ring, OH group and atoms of chlorine. Together with the 19 main compounds, chloroderivatives of methyl- and ethyl-phenols are also considered as CPs. The whole group of CPs comprises tens of compounds, which are significantly differing from each other with respect to their molecular structures and consequently with their physical and chemical properties. With the exception of 2-chlorophenol, all CPs are solids, with melting temperature ranging between 33 and 191 °C. In general, these compounds are weakly dissolved in water, but strongly in organic solvents. Their water solubility decreases with increasing number of chlorine atoms in a molecule. They are weakly acidic and their acidity is slightly higher than that of phenols. In reactions with alkaline non-metals (sodium, potassium) in the aquatic environment, they yield salts highly soluble in water. The physical and chemical properties of selected chlorophenols are presented in Table 1 (Czaplicka 2004).

The fate and transport of a chemical compound in the natural environment strictly depend on the value of the dissociation constant (Ka) and the partition co-efficient ($K_{o/w}$) in the octanol-water system. The dissociation constant depends on the structure of the compound and on the number of chlorine atoms in the molecule. In the case of CPs, the dissociation constant of a compound increases (i.e. its pKa—log Ka decreases) with increasing the number of chlorine atoms in the compound. Depending on the value of pKa, CPs get dissociated totally or partially. Their octanol-water partition co-efficients ($K_{o/w}$) strongly increase with the number of chlorine atoms, and the water solubility (hydrophilicity) inversely decreases. Also, the degree of dissociation of chlorophenols increases (indicated as descending pKa values) with increasing the number of chlorine atoms (Czaplicka 2004).

In addition to being produced commercially, small amounts of some CPs, especially the mono- and dichlorophenols, may be produced when wastewater or drinking water is disinfected with chlorine, if certain contaminants are present in the raw water. They are also produced during the bleaching of wood pulp with chlorine, when paper is being produced. Sorption, volatilization, degradation, and leaching are the primary processes governing their fate and transport in the environment (Ivanciuc et al. 2006).

It has also been reported that physicochemical properties of CPs, such as water solubility, Henry's law constant, organic carbon sorption coefficient, volatilization rate, and photolysis rate determine the transport processes of these compounds. Important factors, influencing these processes include organic matter content and clay content in soil, sediment, and water, as CPs are specially adsorbed to these soil constituents (Czaplicka 2004; Ivanciuc et al. 2006). Generally, as the number of chlorine molecules increases, there is a reduction in the vapor pressure, an increase in boiling point, and a reduction in water solubility of the CPs. Thus, increasing chlorination increases the tendency of these compounds to partition into sediments and lipids and to bioconcentrate (Ivanciuc et al. 2006).

Table 1 Physico-chemicalproperties of CPs (Czaplicka 2004)

No	Compound	Formula	Molecular weight	Boiling point (°C)	Melting point (°C)	Solubility at 20 °C (gl^{-1})	p*K*a	Log $K_{o/w}$
1	2-Chlorophenol	C_6H_5ClO	128.56	174.9	9.3	28	8.3–8.6	2.12–2.17
2	3-Chlorophenol	C_6H_5ClO	128.56	214	33–34	26	8.8–9.1	2.48–2.50
3	4-Chlorophenol	C_6H_5ClO	128.56	217–219	42–44	27	9.1–9.4	2.35–2.44
4	2,3-Dichlorophenol	$C_6H_4Cl_2O$	163.00	206	57–58	NA	6.4–7.8	3.15–3.19
5	2,4-Dichlorophenol	$C_6H_4Cl_2O$	163.00	210	45	4.50	7.5–8.1	2.75–3.30
6	2,5-Dichlorophenol	$C_6H_4Cl_2O$	163.00	211	58–59	NA	6.4–7.5	3.20–3.24
7	2,6-Dichlorophenol	$C_6H_4Cl_2O$	163.00	219	68	NA	6.7–7.8	2.57–2.86
8	3,4-Dichlorophenol	$C_6H_4Cl_2O$	163.00	253–254	65–68	NA	7.4–8.7	3.13–3.44
9	3,5-Dichlorophenol	$C_6H_4Cl_2O$	163.00	233	68	NA	6.9–8.3	2.57–3.56
10	2,3,4-Trichlorophenol	$C_6H_3Cl_3O$	197.45	Sublimes	77-84	0.22	6.5–7.7	3.49–4.07
11	2,3,5-Trichlorophenol	$C_6H_3Cl_3O$	197.45	248–255	57–62	0.22	6.8–7.4	3.84–4.56
12	2,3,6-Trichlorophenol	$C_6H_3Cl_3O$	197.45	246	58	NA	6.0–7.1	3.88
13	2,4,5-Trichlorophenol	$C_6H_3Cl_3O$	197.45	Sublimes	67-70	0.948	7.0–7.7	3.72–4.10
14	2,4,6-Trichlorophenol	$C_6H_3Cl_3O$	197.45	243-249	69	0.434	6.0–7.4	3.60–4.05
15	3,4,5-Trichlorophenol	$C_6H_3Cl_3O$	197.45	271–277	101	NA	7.7–7.8	4.01–4.39
16	2,3,4,5-Tetrachlorophenol	$C_6H_2Cl_4O$	231.89	Sublimes	116–117	0.166	6.2–7.0	4.21–5.16
17	2,3,4,6-Tetrachlorophenol	$C_6H_2Cl_4O$	231.89	150	70	0.183	5.3–6.6	4.10–4.81
18	2,3,5,6-Tetrachlorophenol	$C_6H_2Cl_4O$	231.89	188	114–116	0.100	5.2–5.5	3.88–4.92
19	PCP	C_6Cl_5OH	266.34	300	190	0.014	4.7–4.9	5.01–5.86

NA, not available

4 Distribution, Toxicity and Environmental Fate of Chlorophenols

Chlorophenols are introduced into the environment as effluent waste from the industrial processes, through their use as biocides or as by-products of other industrial operations, such as pulp bleaching with chlorine, water disinfection or even waste burning. Chlorophenols have also been used as general purpose disinfectants, and it has been observed that they can also appear as degradation products of other chlorinated xenobiotics. Many studies have shown that chlorophenols are persistent in water environments, but a few workers have investigated the fate and behavior of these chlorinated aromatics in soil (Lallai and Mura 2004a; Olaniran and Igbinosa 2011). The key source of water pollution by chlorinated phenols is the industrial waste discharge and the leaching of such pollutants from landfills (Zhang and Bennett 2005). Volatile chlorophenols, such mono- and dichlorophenols enter the atmosphere as vapors through volatilization (Ivanciuc et al. 2006). The primary non-point source pollution of chlorophenols comes from the application of pesticides that are made from chlorophenols and the chlorination of wastewater containing phenol (Czaplicka 2004). Monochlorophenols and 2,4-DCP are highly volatile compared to trichlorophenols and tetrachlorophenols, and their volatilization facilitates their diffusion into the atmosphere. However, trichlorophenols and tetrachlorophenols are slightly volatile. Only a small fraction (approximately 5 %) of CPs is emitted to the atmosphere (ATSDR 1999). These releases are primarily in vapor form and are primarily associated with CP production and its use in the manufacture of enduse products (ATSDR 1999). Chlorophenolic compounds are known to be resistant to biodegradation, which makes them persistent in the environment. Because of their lipophilicity, they can be transported through the cell membrane and hence bioaccumulate in the aquatic organisms (Pedroza et al. 2007).

The transformation of CPs in particular could lead to an increase in the toxicity of intermediate compounds or end products due to formation of electrophilic metabolites that may bind and damage DNA or gene products (Michałowicz and Duda 2007). The harmful influence of CPs and their derivatives on the eco-biota may lead to acute toxicity, histopathological changes, mutagenicity, and cancer. These serious health issues make it imperative not only to control CPs in the environment, but also to assess and understand their fate in the environment with a view to protecting the environment and public health.

Cleaning up of contaminated soils by biological processes may be an effective alternative to the chemical technologies in the case of chlorophenols pollution (Al-Thani et al. 2007; Dong et al. 2009). The presence of degraders capable of mineralizing chlorinated aromatic compounds in certain soils are justified by the fact that these compounds are produced naturally (Okpokwasili and Nweke 2005; Potgieter et al. 2009). As the chlorinated phenolic compounds have broad applications in industrial and medical uses, they have become the most common environmental contaminants, especially in the underground water (Breining et al. 2000). CPs get attached to soil and sediments at the bottom of lakes, rivers, or streams and

rapidly enter the body through the skin and the gastrointestinal tract. Owing to their high toxicity, carcinogenicity and wide distribution in industrial wastes they cause harm to human beings and marine organisms (Zhang et al. 2005). Therefore, CPsis of growing concern (Sittig 1981). Therefore, many studies were carried out regarding environmental fate of chlorinated phenols, including photochemical degradation and sequential aerobic and anaerobic degradation. However, bioremediation of CP's has been generally accepted by the public, because they lead to innocuous tailings, are non-invasive to environments and are also cost-effective compared to physiochemical approaches (Gallizia et al. 2003). Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds and the organic pollutants using them as the sole source of carbon and energy by microbes. Generally, chlorinated phenols are transformed via oxidative dechlorination (aerobic biodegradation) (Steinle et al. 1998), while in anaerobic conditions via reductive dechlorination (Annachhatre and Gheewala 1996).

CPs can also be emitted into the environment as waste from sawmills, pulp and paper mills, and due to the incineration of wood wastes (Jones 1983). Industrial effluents can contain all chlorophenols, but 2,4-DCP, 2,4,6-TrCP, and 2,3,4,5-TeCP are detected predominantly (Valo and Salkinoja-Salonen 1986). Some dichlorophenol isomers occur naturally and can be produced by a variety of microorganisms (Siuda 1980). Because of their aromatic structure, phenols are resistant to natural biodegradation due to the difficulty of cleaving of the benzene ring. However, several microorganisms can tolerate phenol and use it as a carbon and energy source (El-Naas et al. 2009). The biological degradation is accomplished through benzene ring cleavage mediated by the enzymes present in the microorganism.

5 Microorganisms to Degrade CPs

Depending on the microbial abilities to grow in specific conditions, organic material can be degraded aerobically or anaerobically (Wang et al. 2007a). A wide range of microorganisms have been shown to be capable of degrading the chloroaromatic compounds. Degradation of the CPs has been demonstrated in the laboratory conditions using both pure cultures and mixed cultures.

CPs degradation studies have been mostly carried out using isolated pure strains in in vitro conditions. Of the strains used, the most common genus is *Pseudomonas,* which has a remarkable potential to evolve entire catabolic sequences for specific degradation pathways. These organisms are ubiquitous in nature and have been found in large numbers in all major natural environments i.e. terrestrial, freshwater and marine. They are also known to have a remarkable nutritional versatility and arecapable of utilizing a wide range of organic compounds as growth substrates. However, a major drawback in biological treatment is the inhibition of microorganisms at high substrate concentrations (Nuhoglu and Yalcin 2005).

The term Pseudomonad is used to describe strictly aerobic, Gram-negative, non-sporulating bacteria. They are oxidase positive with non-acid fast rods, which

are generally straight, but maybe slightly curved, 0.5–1 μm in diameter and 1.5–5 μm in length (Krieg and Holt 1984). They are generally motile with polar flagella and generally do not ferment carbohydrates and fix nitrogen and are also not photosynthesizing. The optimum growth temperature for most strains is 28 °C, but many are capable of growing in the temperature range of 4–43 °C. Members of the genus *Pseudomonas* are free-living organisms and occupy a dominant position in the biosphere in terms of variety of habitats and the number of species in a given habitat. *Pseudomonas* species are sub-divided on the basis of rRNA homology into five similarity groups. Group I is the largest, including scientifically validated relevant strains, such as *P. aeruginosa*, *P. florescence* and *P. putida*. Group I can be further divided into the fluorescent and non-fluorescent species. One of the most striking properties of members of this genus is their remarkable nutritional versatility. They play an important role in the carbon and nitrogen cycles. Organic compounds such as alcohols, aliphatic acids, amides, amines, amino acids, aromatic compounds, carbohydrates and hydrocarbons are all readily used by *Pseudomonas* species as growth substrates. Of these compounds, the aromatic compounds are particularly interesting due to the various pathways by which they are metabolized (Palleroni 1986). The ability of Pseudomonads to utilize a wide range of compounds makes them a vital component of any biodegradative system, particularly in the treatment of wastewaters derived from the chemical industries.

Although both aerobic and anaerobic microorganisms are able to degrade CPs, aerobic processes are generally preferred. Aerobic microorganisms are more efficient for degrading toxic compounds, because they grow faster and usually achieve complete mineralization of toxic organic compounds to inorganic compounds (CO_2, H_2O) (Kim et al. 2002; Kargi and Eker 2004; Sahinkaya and Dilek 2007). For these reasons, there is a limited interest in the utilization of anaerobic bacteria for the degradation of CPs. However, several studies have also been carried out in the anaerobic conditions (Levén et al. 2006; Azbar et al. 2009; Bajaj et al. 2009; Hussain et al. 2010; Puyol et al. 2011; Muñoz et al. 2013).

A large number of CPs degrading bacteria have been isolated and characterized at the physiological and genetic level. The microorganisms used are usually aerobes, such as *Pseudomonas* sp., *Alcaligenes* sp., *Azotobacter* sp., *Rhodococcus* sp. and *Cryptococcus* sp. (Quan et al. 2004; Sahinkaya and Dilek 2007). Pure and mixed cultures of the *Pseudomonas* spp. are the most commonly utilized biomass for the biodegradation of CPs (Stoilova et al. 2007; Caliz et al. 2011). However, *Pseudomonas putida*, has been commonly used for the degradation of phenols due to its high removal efficiency (Hsieh et al. 2008; El-Naas et al. 2010). Responses of *P. putida* to chemical stresses have indicated that its cells could use diverse protective mechanisms for its survival in various extreme environments. These studies could help in synthesizing new bacterial strains with enhanced degradation capability and improved tolerance to toxic pollutants (Loh and Cao 2008).

Although the *Pseudomonas* genus has been widely used to treat chlorophenols, considerable attention has been recently paid to new and more efficient microorganisms for this purpose. *Ralstonia pickettii*, formerly known as *Burkholderia pickettii*, has been reported to have several advantages over other candidate strains

such as *P. putida*, which is only weakly pathogenic. However, no phytopathogenic or animal pathogenic incidents have been reported. *R. pickettii* strain LD1 can metabolize mono-chlorophenols, which poses a challenge as they are particularly formed during the chlorination of wastewaters (Ryan et al. 2007). This aerobic bacterium was found to be able to degrade 2 CP completely (initial concentration: 195 mg L^{-1}), 3 CP (initial concentration: 73.5 mg L^{-1}) and 4 CP (initial concentration: 97 mg L^{-1}) within 30, 30 and 40 h of incubation, respectively, under batch conditions. The rates, at which mono-chlorophenols were degraded, largely depended on the type of chlorophenol: 2 CP > 4 CP > 3 CP (when used as sole carbon sources) (Fava et al. 1995). Al-Zuhair and El-Naas (2012) isolated an indigenous mixed bacterial culture, identified mainly as *R. pickettii*, from oil sludge samples of a local petroleum refinery. Its efficiency for phenol removal was found to be comparable to that of a commercially available mixed culture consisting mainly of *P. putida* (Al-Zuhair and El-Naas 2012).

Fungi share a significant role in the recycling of aromatic compounds in the biosphere. They are capable of consuming a wide variety of carbon sources by enzymatic mechanisms, thus providing possibilities for metabolizing chlorophenols (Stoilova et al. 2007). The most abundant fungi in polluted environments are yeasts. Some yeasts, such as *Candida tropicalis*, *Fusarium flocciferium* and *Trichosporon cutaneum* are capable of utilizing phenols as the major carbon and energy source (Agarry et al. 2008). Moreover, there are studies which confirm the ability of strains from *Penicillium*, *Aspergillus*, *Graphium* and *Phanerochaete* to disintegrate chlorophenolic compounds (Stoilova et al. 2006). Rubilar et al. (2008) analyzed the degradation of CPs by white rot fungi, which are a group of organisms very suitable for the removal of chlorinated phenolic compounds (Rubilar et al. 2008). They are robust organisms which are tolerant to the presence of high concentrations of various pollutants, even with a low bioavailability and this ability is mainly conferred to their very powerful extracellular oxidative enzymatic system (Diez et al. 2012). The first study on the biodegradation of 4 CP by *Fusarium* sp. HJ01 was carried out by Li et al. (2011).

Recently, there has been more interest in evaluating the capabilities of some algae for chlorophenol biodegradation. While some algae have low tolerance to the acute toxicity of chlorophenols, cyanobacteria and eukaryotic microalgae (e.g., *Chlorella* sp., *Scenedesmus* sp., *Selenastrum capricornutum*, *Tetraselmis marina*, *Ochromonas danica*, *Lyngbya gracilis*, *Nostoc punctiforme*, *Oscillatoria animalis* and *Phormidium foveolamm*) are capable of biotransforming phenolic compounds (Lika and Papadakis 2009). Papazi and Kotzabasis (2007) reported that the degradation of phenolic compounds by microalgae seems to be mostly a bioenergetic process, which needs additional energy sources to be exogenously supplied as carbon and light (Papazi and Kotzabasis 2007). Klekner and Kosaric (1992) conducted a study on the degradation of binary phenolic mixtures by *Chlorella*, in which phenol might have induced the proper metabolic pathway of other phenol derivatives (Klekner and Kosaric 1992).

6 Biodegradation of CPs

Biodegradation is based on two processes: growth and co-metabolism. Organic pollutants are used by the microbes as the sole source of carbon and energy during the growth stage, resulting in a complete degradation (mineralization) of organic pollutants. Microbial degradation of chlorophenols has been reported by several researchers (Cortes et al. 2002; Murialdo et al. 2003; Crawford et al. 2007). Chlorophenols are subjected to both abiotic and biotic degradation and conversions (Cortes et al. 2002). Several studies have been carried out on the microbial degradation of chlorophenols in both water and sediments (Demnerova et al. 2005) as well as by sludge (Lallai and Mura 2004a). Even though chlorophenols are poorly biodegradable, several publications have indicated that the aerobic degradation of CPs is possible (Bergauer et al. 2005; Demnerova et al. 2005; Al-Khalid and El-Naas 2013, 2014). The aerobic degradation of chlorophenols by microorganisms requires the involvement of the enzymes i.e. oxygenases, to incorporate atmospheric oxygen into their substrates. The ring is dihydroxylated firstly by an oxygenase in the way that two hydroxyl groups are located either ortho or para to one another in thebenzene ring (Pieper and Reineke 2000). Ring fission occurs subsequently, ingestion of dioxygen atoms into the aromatic nucleus. The vital step in the biodegradation of CPs is the removal of the chlorine substituents. For the catabolism of the lesser substituted phenols (mono- and dichlorophenols), dioxygenase from chlorophenol-degrading bacteria usually opens the dihydroxylated aromatic ring before dechlorination takes place (Farrell and Quilty 2002). However, highly substituted phenols and some of the chloro-substituents must be removed before ring cleavage, as the halogen atoms deactivate the aromatic nucleus to electrophilic attack by dioxygenases (Bergauer et al. 2005). Degradation of 4-chlorophenol is usually accomplished by the oxidation of the substrate to 4-chlorocatechol, followed by ortho cleavage of the aromatic ring (Farrell and Quilty, 2002; Park and Kim 2003). Some microorganisms attack 4-chlorocatechol by meta-cleavage, although it is less common. After ring cleavage, the chlorine atom is removed and the carbon skeleton is transformed into products that are assimilated into the central metabolism of the cell (Bae et al. 1997).

6.1 Biodegradation of CPs in Soil

Lallai and Mura (2004b) reported the biodegradation of 2-CP using microorganisms isolated from the forest soil (Lallai and Mura 2004b). Other studies reported that chlorinated phenols, such as 2-, 3-, and 4-CP; 2,4-, 2,6- and 3,4- DCP; 2,4,6-, and 2,4,5-TCP; and PCP, were incubated under aerobic conditions and degraded with a Canadian clay loam grassland soil (Baker and Mayfield 1980); whereas, 3,4,5-TCP and 2,3,4,5-TeCP were not degraded. Chlorophenolic compounds with ortho and para- substituted chlorines were reported to be totally degraded within a few days.

Kiyohara et al. (1989) reported that 26 out of 170 soil samples were found to degrade 2,4,6-TCP, indicating a wide distribution of CPs degraders in the environment (Kiyohara et al. 1989). High concentrations up to 5000 mg kg^{-1} of 2,4,6-TCP were easily degraded by CP free forest soil (Sanchez et al. 2004). It was also observed that soil microbial structure was not changed by doses of 2,4,6-TCP as high as 500 mg kg^{-1}. PCP at concentrations of 200 mg kg^{-1} was reported to be degraded by local microorganisms in unspoiled grassland soils at an average rate of 1.2 mg $kg^{-1}d^{-1}$ (Mahmood et al. 2005). Molecular fingerprinting of the soil microbial population was utilized to demonstrate the enhancement of PCP-degrading strains of bacteria. Degradation of PCP was also observed in Japanese soils, where it had been used as an herbicide (Watanabe 1978). Reddy and Gold (2000) reported that five of ten wetland soil samples from five states in the USA caused PCP to be biodegraded, and about 75-100 % of the PCP was removed in 30 days (Reddy and Gold 2000).

6.2 Biodegradation of CPs in Sediments

Aerobic degradation of chlorinated phenols occurred in surface water samples, where radio labeled 3,4-DCP, 2,4,5-TCP and PCP were mineralized into CO_2 in two types of lake water samples (Larsson and Lemkemeier 1989). Surface water from an estuarine river degraded 4-CP (Hwang et al. 1986). Mississippi river channels, exposed for several weeks to PCP, developed microbial populations responsible for the biological mineralization of PCP to CO_2. This degrading activity was attributed to microorganisms attached to rock or water plant surfaces (Pignatello et al. 1983). The biodegradation of 4-CP, 2,4-DCP, 2,5-DCP, 3,4-DCP and 2,4,5-TCP in pool sediments and 4-CP, 2,4-DCP and 2,5-DCP in methanogenic aquifer sediments was observed (Gibson and Suflita 1986). They also reported that phenols and lower chlorinated phenols were detected as intermediate compounds of CP degradation.

The bioconversion of 2-CP was also observed in Lake Michigan sediments (Becker et al. 1999). All isomers of mono-chlorinated phenol were degraded by river sediments of the upper Hudson River in New York State under methanogenic and sulfate reducing conditions (Haggblom et al. 1993) and phenol, benzoate and 3-chlorobenzoate were observed as intermediates of the biotransformation. Susarla et al. (1997) measured the kinetics of anaerobic degradation of all 6 DCP isomers in lake sediments in Japan and determined the first-order rate constants for 3,4-DCP and 2,5-DCP. Monochlorophenol and phenol were invariably observed as intermediates in the degradation process. Three isomers of DCP, 2,3-DCP, 2,4-DCP and 2,6-DCP were anaerobically degraded with first-order rate constants of 0.017–0.033 and 0.087–0.139 d^{-1}, respectively; for two different chemicals free pond sediments collected in the Southeastern U.S. Lake sediment samples from the Southeastern U.S. were also shown to anaerobically degrade 2,4-DCP with the formation of 4-CP as a main intermediate in the process (Kohring et al. 1989; Zhang and Wiegel 1990).

The first-order rate constants of PCP degradation ranged from 0.082 to 0.38 d^{-1}. Tetrachloro-1,4-hydroquinone was dehydroxylated and dechlorinated by a mixed culture derived from Baltimore harbor sediments to yield 2,3,5-TCP (Milliken et al. 2004). Anoxic estuarine sediments from Taiwan were found to degrade2-CP, 3-CP, 4-CP, 2,5-DCP, 3,4-DCP, 3,5-DCP and PCP (Liu et al. 1996). PCP degradation was linked to sulfate reduction. Estuarine sediments from the lower Hudson River in New York were able to degrade 2-CP, 3-CP and 4-CP under sulfate-reducing conditions (Uotila et al. 1992). Estuarine sediments from the Chesapeake Bay biotransformed 2,4-DCP to 4-CP when incubated under methanogenic conditions (Warner et al. 2002). Similarly, 2,4-DCP and 3,4- DCP were dechlorinated by marine sediments to 4-CP and 3-CP, respectively (Boothe et al. 1997). Marine sediments collected off the coast of Sweden were observed to dechlorinate PCP, yielding 2,3,4,5-TeCP, 3,4,5-TCP and 3,5-DCP (Abrahamsson and Klick 1991).

6.3 Biodegradation of CPs by Sludge

Municipal anaerobic digester sludge has also been used to degrade chlorophenols. All monochlorinated isomers were degraded by anaerobically-digested primary sewage sludge (Boyd and Shelton 1984), and all dichlorophenols (except 3,4-DCP and 3,5-DCP) were degraded via monochlorophenol intermediates. Municipal sludge, from the anaerobic digester dechlorinated 2-CP to phenol. Further, evaluation of selective inhibitors demonstrated that the population responsible for the dechlorination reaction was associated with syntrophic acetogens (Basu et al. 2005). The ability of municipal anaerobic digester sludge to degrade 12 chlorinated phenolic compounds was tested and 10 of the compounds were found to be degraded (Loh and Wang 1998). However, the digested sludge culture, did not degrade PCP or 2,3,4,6-TeCP. In another study, all chlorinated phenol isomers were degraded by methanogenic sludge with first-order rate constants of 0.00046–0.161 d^{-1} (Takeuchi et al. 2000). They reported that dechlorination of ortho-chlorines occurred at the fastest rate; whereas dechlorination of para-chlorines occurred at the slowest rate. First-order rate constants decreased with decreasing number of chlorine substituent.

7 Mechanisms of CPs Biodegradation

Under aerobic conditions, chlorinated aromatic compounds persist in the environment because chlorine atoms interfere with the action of many dioxygenase enzymes that normally initiate the degradation of aromatic rings. The interference is likely to be due to both steric and electronic effects, because chlorine atoms are both larger and more electron-withdrawing than hydrogen atoms. Despite these problems, microorganisms that can degrade chlorinated aromatic compounds, can often

be found at highly contaminated sites. Many of these microorganisms contain dehalogenase enzymes that catalyze the removal of chlorine atoms from the aromatic ring. Once the chlorine atoms are removed, the aromatic compound becomes susceptible to microbial degradation.

Chlorinated aromatic compounds are also degraded under anaerobic conditions. For example, the PCBs in the sediments of the Hudson River are gradually being converted to the lesser chlorinated congeners (Brown et al. 1987). The pathways of anaerobic degradation of chlorinated aromatic compounds are less extensive than that of pathway for aerobic degradation, however. Furthermore, mechanistic studies of an aromatic dehalogenase from an anaerobic microorganism have never been carried out. Chlorine substituents can be removed from aromatic rings in three fundamentally different ways as illustrated in Fig. 2. Hydrolytic dehalogenases replace chlorine substituents with hydroxyl groups that are derived from water; reductive dehalogenascs replace chlorine substituents with hydrogen atoms; and oxygen-dependent dehalogenases replace chlorine substituents with hydroxyl groups whose oxygen atoms are derived from O_2. Although only a limited number of dehalogenascs have been studied, it is already evident that there is more than one way to accomplish each of three classes of reactions. The mechanisms used by enzymes in each of these three classes evidently show the diversity of mechanisms found in nature and the relationships between dehalogenase enzymes and other known enzymes (Copley 1997).

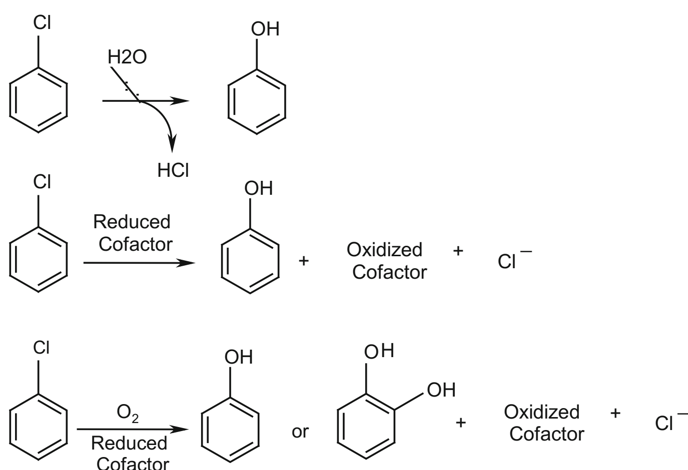


Fig. 2 Different processes for the removal of chlorine substituent from aromatic compounds (Copley 1997)

7.1 Aerobic Biodegradation of CPs

Aerobic degradation of phenol occurs via the formation of catechol. The bacterium, *Pseudomonas putida*, utilizes phenol through meta-oxidation and the yeast, *Trichosporon cutaneum*, operates through ortho oxidation (Yang and Humphrey 1975; Al-Khalid and El-Naas 2011). Haloaromatic compounds are known to be degraded through the formation of halocatechols as intermediates, which are subsequently cleaved by dioxygenases. This is followed by dehalogenation through the elimination of the hydrogen halide, with subsequent double bond formation on the aliphatic intermediate (Annachhatre and Gheewala 1996).

Removal of chlorine can be achieved in three fundamentally different ways in which the dehalogenases replace chlorine substituent with (a) hydroxyl groups derived from water, (b) hydrogen atoms, and (c) hydroxyl groups whose oxygen atoms are derived from O_2 (Copley 1997).

Research on the degradation of phenols and CPs in the aerobic soil environment revealed that phenol, ochlorophenol, 2,4-DCP, 2,6-DCP and 2,4,6-TCP could be rapidly degraded as compared to PCP, 2,4,5-TCP, 3,4-DCP and m-chlorophenol, whereas 3,4,5-TCP and 2,3,4,5-TeCP persisted in the soil for over 160 days (Baker and Mayfield 1980). Chlorine attachment in the meta-position, toxicity to soil microorganisms and unavailability of compounds to microbes due to their adsorption on soil were some of the possible reasons for their reduced biodegradability. However, satisfactory degradation of phenol, monochlorophenols, dichlorophenols, trichlorophenols and PCP in a fine sandy loam soil was observed (Namkoong et al. 1988). Likewise, phenol and PCP were degraded easily in lake waters and fresh water streams, respectively (Pignatello et al.1983; Annachhatre and Gheewala 1996).

The degradation of CPs by aerobic bacteria using two main strategies was reported by Solyanikova and Golovleva (2004). Lower chlorinated phenols are initially attacked by monooxygenases yielding chlorocatechols as the first intermediates (chlorocatechol pathway), which are subjected to ring cleavage prior to dechlorination. On the other hand, polychlorinated phenols are converted to chlorohydroquinones as the initial intermediates (hydroquinone pathway). Subsequent reactions gradually remove chlorines from the ring prior to its cleavage (Solyanikova and Golovleva 2004). The chlorocatechol pathway is illustrated for the chlorophenol, 2,4-DCP, as shown in Fig. 3.

The 2,4-DCP attack is initiated by a monooxygenases forming 3,5-dichlorocatechol (Finkel'shtein et al. 2000). The chlorocatechol is ortho cleaved, yielding 2,4-dichloromuconic acid (Tiedje et al. 1969). The first dechlorination takes place when a lactonizing enzyme converts the dichloromuconic acid to 2-chloro-4-carboxymethylenebut-2-enolide (Sharpee et al. 1973). The degradation of PCP is initiated by the formation of tetrachlorohydroquinone (TeCHQ) because of the hydroxylation at the para-position by either PCP-4-monooxygenase (Orser and Lange 1994) or cytochrome P-450 type enzyme (Uotila and Salkinoja-Salonen 1991; Uotila et al. 1992). In *Sphingomonas chlorophenolicum*

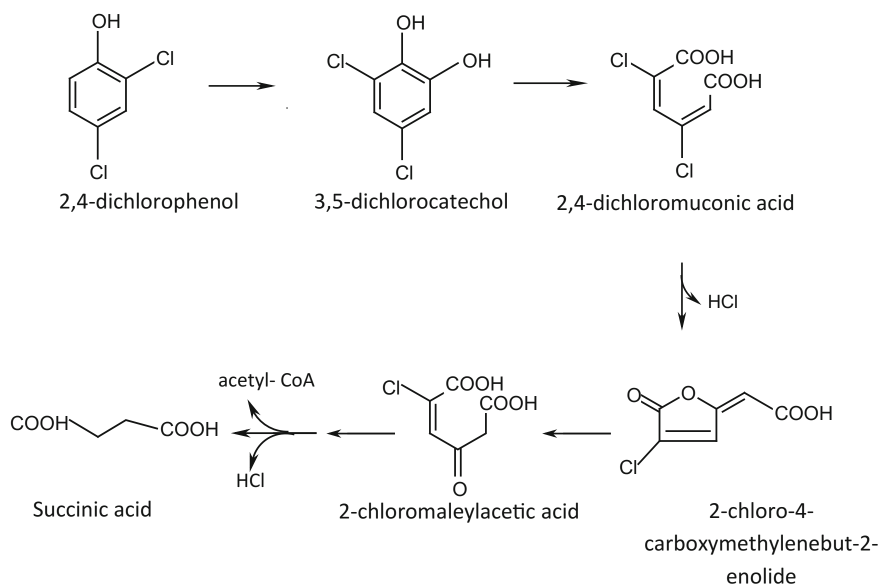


Fig. 3 Proposed pathway of the aerobic degradation of 2,4-dichlorophenol by bacteria (Field and Sierra-Alvarez 2008)

L-1, PCP-4-monooxygenase (PcpA) catalyzes the conversion of PCP to TeCHQ via the removal of chloride ions (Orser and Lange 1994). In the next step, TeCHQ is consecutively dehalogenated to 2,6-dichloro- 1,4-hydroquinone (2,6-DCHQ) through a TeCHQ-reductive dehalogenase. Further degradation of 2,6-DCHQ happens via ring cleavage by the 2,6- DCHQ-1,2-dioxygenase, leading to the formation of 2-chloromaleylacetate, which is terminally degraded via the TCA cycle (Arora and Bae 2014, Fig. 4a).

In *Mycobacterium chlorophenolicum* PCP-1 and *Mycobacterium fortuitum* CG-2 (formerly *Rhodoccocus* strains), PCP is hydroxylated to TeCHQ by a membrane bound cytochrome P-450 type enzyme (Uotila et al. 1992); then, TeCHQ goes through hydrolytic dehalogenation followed by reductive dehalogenation to procedure dichloro-1,2,4-trihydroxybenzene (Apajalahti and Salkinoja-Salonen 1987) which yields BT after two successive reductive dehalogenation as represented in Fig. 4b (Arora and Bae 2014).

Different pathways of the aerobic degradation of chlorophenol have been reported. For example, the degradation of 4-CP by *Arthrobacter ureafaciens* strain CPR706 and *Arthrobacter chlorophenolicus* strain A6 is initiated by dechlorination, yielding 1,4-hydroquinone as the intermediate (Bae et al. 1997; Nordin et al. 2005). The proposed pathway involves the conversion of 4-CP to hydroquinone, to hydroxyquinol and then to maleylacetate (Nordin et al. 2005). A ring cleaving hydroxyquinol dioxygenase is a key enzyme of this pathway. When a gene coding for the dioxygenase is disrupted, *Arthrobacter chlorophenolicus* has a negligible

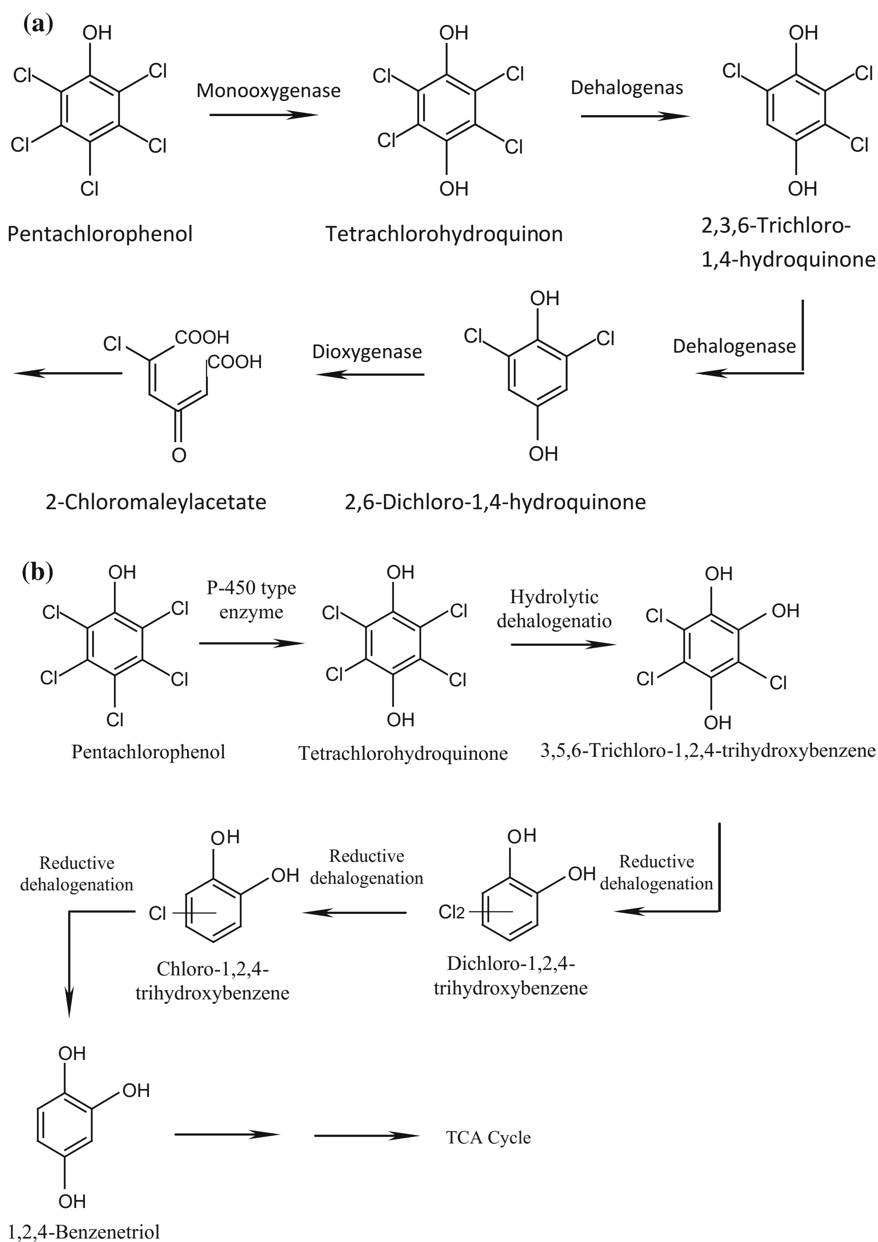


Fig. 4 **a** Proposed pathway of the aerobic degradation of pentachlorophenol by *Sphingomonas* bacteria (Arora and Bae 2014)**.** **b** Proposed pathway of the aerobic degradation of PCP by *Mycobacterium* bacteria (Arora and Bae 2014)

growth on 4-CP (Nordin et al. 2005). The monooxygenase from *Ralstonia eutropha* strain JMP134 (pJP4) catalyzes successive dechlorination reactions of 2,4,6-TCP to 2,6-dichlorohydroquinone and then to 6-chlorohydroxyquinol prior to ring cleavage by a hydroxyquinol dioxygenase (Matus et al. 2003).

A unique property of the monooxygenase is that the second dechlorination reaction does not require O_2, instead the dechlorination of 2,6-dichlorohydroquinone is catalyzed by a hydrolytic activity associated with the monooxygenase (Xun and Webster 2004). Some aerobic bacteria implicated in the rapid degradation of higher chlorinated phenols are known to convert CPs into methylated biotransformation products. *Mycobacterium* strains were known to O-methylate a variety of CPs (Haggblom et al. 1988b). The preferred substrates of the methylation were hydroxyl groups doubly flanked by chlorine groups.

Chlorinated hydroquinones were readily O-methylated (Haggblom et al. 1988a) accounting for the occurrence of methylated chlorohydroquinone intermediates during PCP biodegradation (Suzuki 1983). Direct methylation of PCP resulted in the formation of pentachloroanisole in soil (D'Angelo and Reddy 2000).

7.2 Anaerobic Biodegradation of CPs

In anaerobic environments, the biodegradation of chlorinated aromatic takes place through reductive dehalogenation (Horowitz et al. 1983) leading to the formation of less toxic and more biodegradable compounds (Gibson and Suflita 1986; Mohn and Kennedy 1992). Reductive dechlorination of 2,4-DCP was followed by carboxylation, ring fission and acetogenesis, and methanogenesis, which led to the complete mineralization of 2,4-DCP as illustrated in Fig. 5 (Zhang and Wiegel 1990). 2,4-DCP was also biodegraded to 4-CP in anaerobic sediments (Boyd and Shelton 1984).

Fig. 5 Anaerobic mineralization of 2,4-dichlorophenol (Zhang and Wiegel 1990)

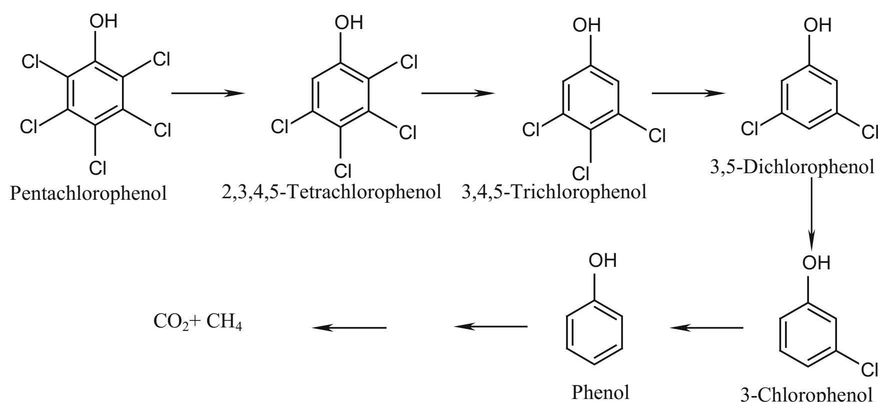


Fig. 6 Anaerobic dehalogenation and degradation of PCP (Arora and Bae 2014)

The reductive dehalogenation is a critical step for the anaerobic biodegradation of CPs, especially for poly-CPs. Several poly-CPs resist the aerobic bacterial attack and can be reductively dehalogenated into chlorinated phenols of less number of chlorine atoms which can be mineralized easily by anaerobic bacteria (Mikesell and Boyd 1986; Londry and Fedorak 1992, Fig. 6).

The presence of phenol-dehalogenating and phenol-degrading bacterial cultures together in a system enhanced the anaerobic mineralization process of PCP (Yang et al. 2009). In this process, a phenol-dehalogenating culture dehalogenates PCP to phenol under anaerobic conditions. The phenol is then further degraded by phenol-degrading bacterial culture under iron reducing or sulfate reducing conditions (Yang et al. 2009).

Suitable biodegradation of phenol, 2-CP, 2,4-DCP, 2,4,6-TCP and PCP under anaerobic conditions in the soil was observed by Smith and Novak (1987). The biodegradation rates followed the order: phenol = 2-CP > 2,4,6-TCP > 2,4-DCP. The PCP degradation rate was slower than that of phenol or 2-CP, but comparable to 2,4,6-TCP and 2,4-DCP. This suggests that increased chlorination of the aromatic ring does not necessarily have a direct correlation with resistance to biodegradation. Rates of degradation also varied with soil characteristics. Groundwater nitrate concentration was suggested as a possible reason for the difference in the degradation rate of chlorophenols (Annachhatre and Gheewala 1996).

7.3 Co-Metabolic Transformation of CPs

The first definitive report on the co-metabolic study appeared in the late 1950s (Kim and Hao 1999). A strain of *Pseudomonas methanica*, growing with methane (growth substrate) as a sole source of carbon and energy, was shown to oxidize other alkanes such as ethane and propane (non-growth substrate) to their

corresponding fatty acids. Since then, many other co-metabolizing cultures have been identified (Alvarez-Cohen and McCarty 1991). A large number of chlorinated solvents can be degraded under aerobic conditions by means of co-metabolic transformation reactions (Alvarez-Cohen and Speitel 2001). Co-metabolism of monochlorinated phenols with phenol has been shown to be an effective way for the biodegradation of these toxic compounds. The *Rhodococus* species can co-oxidize aniline, phenol and their monochlorinated isomers in the presence of glucose or acetate (Janke et al. 1989; Dapaah and Hill 1992). The degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes* sp. A7-2 was evaluated by Menke and Rehm (1992), and it was found that 3-CP could only be mineralized co-metabolically. It was also reported that *Acinetobacter* species could also transform monochlorophenols via co-metabolism (Kim and Hao 1999; Hao et al. 2002). *Pseudomonas putida* was also utilized to degrade phenol and monochlorinated phenols by means of cometabolic transformation (Loh and Wang 1998).

The difficulties of co-metabolism started with the description of the process (Semprini 1997). A general consensus refers to co-metabolism as the metabolism of a substrate not required for growth in which no apparent benefit is accrued by the metabolizing organism (Horvath 1972). Therefore, a growth substrate must be available at least periodically to support the cell growth. For example, 4-CP cannot serve as the sole carbon and energy source for the growth of *Pseudomonas putida*, but when phenol is present, they can be transformed together, with phenol as the growth substrate and 4-CP as the non-growth substrate. One main reason for such widely observed microbial co-metabolism is the lack of specificity of existing enzymes and co-factors. Therefore non-growth substrate with a similar structure to that of growth substrate could bind to enzymes and be transformed. Especially, oxygenase enzymes have shown non-specific activities towards non-growth substrates, such as chlorinated solvent (Kim and Hao 1999). The oxygenase reaction can oxidize the chlorinated compounds to intermediates that may be hydrolyzed spontaneously into carbon dioxide, chloride or other products that can be easily attacked by the microorganisms (Alvarez-Cohen and Speitel 2001).

The addition of conventional carbon source was also taken to augment the performance of co-metabolic degradation (Wang 1997). In spite of the rather extensive investigation of co-metabolic transformation, more studies (not only for qualitative perspective) were found of great practical significance, due to the complex nature of co-metabolism and its effectiveness as a treatment method of chlorophenols waste.

8 Factors Affecting the Biodegradation of CPs

Degradation rates are strongly influenced by a wide variety of environmental factors which affect microbial activity. The bacteria or their enzymes must be in contact with the pesticide, and the pesticide or its metabolites must be transported into the

cell to continue the degradation. Some bacteria display chemical responses to substrates, which may play an important role in the environment, while others grow in a filamentous form towards potential substrates (Fewson 1988). The environmental factors include presence of appropriate microorganisms, contact between the microbe and the substrate, pH, temperature and salinity, available water, oxygen tension and redox potential, nutrient availability, presence of alternative carbon substrates, light quality and intensity, binding to surfaces and alternative electron acceptor etc. However, molecule factors include chemical structure, molecular weight and functional groups concentration and toxicity, and solubility in water.

8.1 Effect of PH

The pH and alkalinity have important roles to play in the biological treatment of wastewater. Extreme pH values of the medium (less than 3 or greater than 9) as well as sudden changes in pH in which the microbe is present, can inhibit its growth (Agarry et al. 2008). In aerobic biological oxidation, pH in the range of 6.0–9.0 is suitable (Majumder and Gupta 2009). Therefore, laboratory studies on chlorophenols degradation are usually carried out at or near neutral pH values (pH 7.0). However, Alva and Peyton (2003) reported that a haloalkaliphilic bacterium, *Halomonas campisalis*, is able to thrive in both saline and alkaline environments, such as soda lakes. The bacterium degraded phenol and catechol both in alkaline (pH values of 8–11) and saline environments (0–150 g L^{-1} NaCl). This is specifically significant for an industrial effluent of a petroleum refinery containing spent caustic, which is an alkaline and saline waste stream that contains phenol as well as other aromatic compounds (Alva and Peyton 2003).

Many investigators have noted that pH plays an important role in the adsorption of compounds with acidic functional groups on activated carbon and soil, because the neutral and ionic forms display very different adsorption properties (Kookana and Rogers 1995; Diez et al. 1999; Cea et al. 2007). However, CPs sorption in soil has been generally described assuming hydrophobic partitioning of the neutral species to soil organic matter (DiVincenzo and Sparks 2001). Diez et al. (1999) demonstrated that in a variable-charged soil, the adsorption of phenolic compounds from Kraft mill effluent increased with decreasing pH, possibly as a consequence of electrostatic repulsions between the organic compound and the resulting negative surface charge as the pH increases (Diez et al. 1999).

8.2 Effect of Temperature

Temperature is another important parameter that is known to have a significant effect on the growth and the biodegradation capabilities of microorganisms. Conventionally, organisms can be divided into psychrophilic and psychrotrophic.

Psychrophiles are the organisms that have minimum, optimum and maximum growth temperatures of $\leq$ 0, $\leq$ 15, $\leq$ 20 °C, respectively. The corresponding temperatures for psychrotrophsare 0–5, > 15 and > 20 °C. It is evident that the most interesting organisms are often the psychrotrophs, since they are also active at temperatures above 20 °C (Li et al. 2010). Therefore, temperature-dependent performance of biological processes may be strongly influenced by their content of psychrotrophic bacteria. Most of the studies on CP biodegradation had been carried out in the temperature range 25–30 °C. Li et al. (2011) reported that there has been little focus on microorganisms that function at low temperature. Therefore, it is necessary to find a kind of organism that can adapt to the daily and seasonal fluctuations in the environmental temperatures, with a focus on cold-adapted microorganisms in environments where they are needed. The bacterial strain *P. putida* LY1, as a psychrotrophic microorganism, was shown to grow on phenol as the sole carbon and energy source and survive well in a wide range of temperatures (Li et al. 2011). On the other hand, only a few studies have focused on the biodegradation of phenols by thermophilic or hyperthermophilic microorganisms, although many phenolic effluents are very hot. Such microorganisms can be robust biocatalysts with potential applications (Christen et al. 2012). A strain of *P. putida* has been successfully used to degrade phenol at low temperature of 10 °C, while the bacterium *Bacillus stearothermophiles* was found to effectively degrade phenol at 50 °C (Agarry et al. 2008). Several studies on these aspects have been documented in this regard (Christen et al. 2012; Margesin et al. 2013).

Thus, temperature significantly affects critical process and design parameters, such as the critical dilution rate, which corresponds to the limit above which biomass washout occurs and, hence, failure of the biological treatment process (Onysko et al. 2000). According to Onysko et al. (2000), there is a lack of sufficient information about the change of microbial kinetics as a function of temperature. The authors attempted to model the temperature dependence of cell growth and phenol degradation kinetics of the psychrotrophic bacterium *P. putida* Q5 in both batch and continuous cultures in the temperature range of 10–25 °C (Onysko et al. 2000).

8.3 Effect of Substrate Concentration

High concentrations of chlorophenols are usually inhibitory for the microorganisms. However, pre-adaptation or acclimatization of microorganisms to chlorophenols improves their biodegradation capability and alleviates inhibition effects to some extent (Tobajas et al. 2012). Sometimes, due to substrate inhibition, combined processes have been used to treat highly loaded wastewaters, such as the sequential UV-biological degradation of a mixture of chlorophenols and sequential anaerobic-aerobic reactors (Armenante et al. 1999; Tamer et al. 2006; Majumder and Gupta 2007). Wang et al. (2007b) indicated that there is a little information on bacteria with a high tolerance to phenols and high metabolizing activity. Therefore,

there is still a need to isolate new phenols-degrading bacteria that can grow at elevated concentration of phenol (Wang et al. 2007b).

The ability of microbial communities to degrade pollutants is affected by the presence of naturally occurring carbon sources. In general, adaptation to variations in the concentration of nutrients, such as glucose, yeast extract and $(NH_4)_2SO_4$, enhances the ability to degrade phenols. However, it was observed that different CPs may give different responses to the presence of biogenic substrate (Sahinkaya and Dilek 2006a, b). The rate of degradation largely depends on the composition of the medium which affects the degradation efficiency. Shourian et al. (2009) found that phenol-containing minimal medium, supplemented with mannitol and casein, showed the highest degradation rate among all carbon and nitrogen nutrients tested. The highest inhibitory effects were observed with the addition of sucrose and glycerol. In another study, Stehlickova et al. (2009) observed higher biodegradation rate and more intensive growth during the cultivation in the presence of potassium humate (humic substance) than the cultivation without its addition. This effect of humic substances was also confirmed by Hahn et al. (2007) indicating a reduction in the 2,4 DCP toxicity to *P. putida* after oxidative incubation with humic substances.

9 Activities of Microbes in Soils Contaminated with CPs

Both indigenous and exogenous microorganisms are responsible for the degradation of pesticides in soils during the composting process. The presence of pesticides affected the population and activity of microbes in contaminated soil with composting. The interaction between contaminant and microbes also influences the effect of composting on soil remediation. Contaminant toxicity to microbes also affects the biodegradation of some pollutants. The undesirable properties of contaminants have adverse effects on the growth of microorganisms by interfering with their normal metabolic function. Studies on the dynamics of microbial community during composting of PCP-contaminated soils revealed that microbial abundance was inhibited by PCP stress (Ren et al. 2011). In a study carried out by (Scelza et al. 2008), the soil microflora was found very difficult to recover from its inhibitory state induced by the toxicity of PCP. Composting exhibited a strong effect on the removal of CP with a degradation potential of 85.85 % (decomposed from 212 to 30 mg kg^{-1}) in four summer months. After the second summer of composting, the biodegradation resulted in the concentration of chlorophenols to only 15 mg kg^{-1} (Valo and Salkinoja-Salonen 1986). Bacterial populations, which occurred in compost, manure and cornstalk-amended soils at a rate of 5 %, were increased significantly compared to non-amended soils, but these amendments had no effect on fungal or actinomycete populations (Moorman et al. 2001).

The addition of pollutant-degrading microorganisms can certainly improve the degradation progress, but a large number of microbial degraders do exist in the composting of contaminated soil. In the presence of *Rhodococcus chlorophenolicus* that can degrade several types of chlorophenols as amendment, the decomposition of chlorophenols was faster than that without the inoculation of this microorganism (Valo and Salkinoja-Salonen 1986). *P. chrysosporium*, known as a type of basidiomycete, exhibited high degradation ability for lignin, PCP, and others by secreting lignin peroxidase (LiP) and manganese peroxidase (MnP) (Martinez et al. 2005; Yu et al. 2011). Thus, *P. chrysosporium* inoculants could increase lignocelluloses biodegradability and ultimately improve the quality of compost products. Yu et al. (2011) showed that the inoculation of *P. chrysosporium* had a strong positive effect, both on the composting efficiency and the removal of PCP. This study also indicated that inoculation time influenced the removal of PCP in soil based on composting, because the inoculation on the fifteenth day of composting had a better effect on PCP removal than that in the beginning. Thus, the positive effect of microorganism addition on remediation of polluted soil with composting cannot be neglected. However, mineralization of pollutants in soil by microbes in composting may also pose ecotoxicological risks to the soil environment, if incomplete pollutant mineralization occurs (Laine and Jørgensen 1997). As suggested by Laine and Jørgensen (1997), safe, effective and fast composting methods with complete mineralization needed to be developed. White rot fungi have been shown to have a high capacity to remove various organic pollutants during composting. However, sometimes, white rot fungi also convert organic contaminants to harmful metabolites. For example, white rot fungi may transform CS into PCDD/Fs by peroxidase enzymes (Laine and Jørgensen 1997; Oberg and Rappe 1992).

The application of composting technology as a remediation strategy for contaminated soil requires an understanding of microbes involved in pollutant biodegradation and biotransformation. The knowledge about the effect of more types of pollutant-degrading microorganisms as amendments during composting of contaminated soil is still poor despite the availability of various degrading microorganisms. A number of microorganisms can grow on the chlorophenols compounds using them as a sole source of carbon and energy. A list of typical microbes that can degrade CPs has been prepared by Gaofeng et al. (2004) as reflected in Table 2.

As indicated above, *Pseudomonas* spp. can dechlorinate various CPs in suspension cultures. In fact, *Pseudomonas* spp. can also degrade many other aromatic compounds, such as other chlorinated aromatic compounds, nitrified aromatic compounds, aminophenols, and polycyclic aromatic hydrocarbon (An et al. 2001). *Desulfomonile tiedjei*, a strictly anaerobic Gram-negative sulfate-reducing bacterium, is the best-described dechlorinating anaerobic bacterium to date (Middeldorp et al. 1997).

Table 2 Common chlorophenols-degrading microbes (Gaofeng et al. 2004)

Chlorophenols	Microbes
2-chlorophenol	*Desulfovibrio dechloracetivorans* (ATCC700921), *Alcaligenes* sp., *Ralstonia* sp., *Azotobacter* sp., *Pseudomonas putida*, *Cystobacteri* sp., *Ps. cepacia*
3-chlorophenol	*Desulfomonile tiedjei*
4-chlorophenol	*Ps. putida, Comamonas testosteroni* JH5, *Ps. cepacia*, *Rulstonie eutropha, Alcaligenes* sp., *Azotobacter* sp., *Ralstonia* sp.
2,3-dichlorophenol	*Desulfitobacterium dehalogenans* (JW/IU-DC1), *Desulfomomile tiedjei*
2,4-dichlorophenol	*Desulfitobacterium dehalogenans* (JW/IU-DC1), *Desulformonile tiediei*, *Ralstonia* sp., *Clostridium* sp., *Burkholderia cepacia, Pseudomonas pickettii* (DTP0606)
2,5-dichlorophenol	*Desulfomonile tiedjei, Desulfovibrio dechloracetivorans*
2,6-dichlorophenol	*Desulfitobacterium dehalogenans* (JW/IU-DC1), *Mycobacterium chlophenolicum, Ps. cepacia, Azotobacter* sp., *Ps. pickettii* (DTP0606), *Desulforibrio dechloracetivorans, Ralstonia* sp.
3,4-dichlorophenol	*Ps. pickettii* (DTPO602)
3,5-dichlorophenol	*Clotridium* sp., *Desulfomonile tiedjei*
2,3,4-trichlorophenol	*Desulfovibrio dechloracetivorans* (JW/IC-DC1), *Ps. pickettii* (DTPO602)
2,4,6-trichlorophenol	*Ps. pickettii* (DTPO602), *Azotobacter* sp., *Desulfitobacterium dehalogenans* (JW/IU-DC1), *Clostridium* sp., *Phanerochaete chrysosporium*
2,4,5-trichlorophenol	*Clostridium sp., Ps. pickettii* (DTPO602)
Tetrachlorophenols	*Ps. pickettii* (DTPO602), *Ralstonia* sp., *Arthrobacter* sp.
Pentachlorophenol	*Flavobacterium* sp., *Desulfomonile tiedjei* sp., *Clostridium* sp., *Rhodococcus chlorophenolicus, Desulfitobacterium frappier* (PCP-1), *Desulfitobacterium dehalogenans* (JW/IU-DC1), *Ps. cepacia* (AC110)

10 Involvements of Enzymes in Degradation of CPs

The enzymes in the microbial degradation of CPs are categorized mainly as the oxygenases and dioxygenases (Olaniran and Igbinosa 2011). Based on the literature describing CP biodegradation pathways, anaerobic degradation of CP includes reductive dehalogenation as the first step with the favored replacement of chlorine by hydrogen from ortho- position. The aerobic degradation of CPs may occur either through the ortho-pathway or the meta- pathway, following their conversion to chlorocatechols (Solyanikova and Golovleva 2004; Majumder and Gupta 2007). Thus, catechol is a central intermediate in the aerobic degradation pathways of various aromatic compounds. Fava et al. (1995) suggested the chlorocatechol pathway in their investigation of aerobic degradation of different mono-chlorophenols by a *Pseudomonas pickettii* strain. However, it has been reported in the literature that aerobic biodegradation of mono and dichlorophenols follows mainly the ortho-cleavage pathway after the formation of chlorocatechol,

because the meta- cleavage produces toxic products from chlorocatechol (Lu et al. 1996; Solyanikova and Golovleva 2004).

Conversion of chlorocatechol as a rule does not proceed via meta- cleavage of the aromatic ring, as catechol dioxygenase enzyme is inactivated by the accumulation of an intermediate, which is toxic to cells. Meta-cleavage of chloroaromatics generally results in the formation of dead-end metabolites. In general, the ortho-cleavage pathway is required for the complete degradation of mono and di-chlorophenols. Only meta- cleavage pathways have been observed for chlorocatechols (Ferraroni et al. 2006). *P. putida* CP1 was added to a commercial mixed culture that degraded 2 CP. The augmented mixed culture degraded 2 CP using an ortho-cleavage pathway, whereas previous degradation of the mixed culture occurred using the meta-cleavage pathway.

Metabolic processes are governed by the action of enzymes, which are specific for each type of reaction. Thus microbial metabolism is a process of energy conversion sustained by specific reactions and provides the ultimate source of energy (Agarry et al. 2008). The biodegradation process requires the presence of molecular oxygen to initiate an enzymatic attack on the aromatic rings. A typical pathway for metabolizing phenols is to hydroxylate the ring by the enzyme phenol hydroxylase, form catechol and then open the ring through ortho- (also termed β-ketoadipate pathway) or meta-oxidation (Jiang et al. 2006; Lika and Papadakis 2009). Phenol hydroxylase represents the first enzyme in the metabolic pathway of phenol degradation. Both ortho-pathway and meta-pathway are distinguishable by evaluating their characteristic enzyme activities. In the ortho-pathway, the aromatic ring is cleaved by the enzyme catechol 1, 2 dioxygenase (C12O). In the meta-pathway, the ring is cleaved by the enzyme catechol 2, 3 dioxygenase (C23O). Therefore, the ring is first opened and then degraded (Agarry et al. 2008). The ring cleavage occurs in two different directions and this difference in cleavage site is used to classify catechol dioxygenases in two groups: the intradiol (such as C12O) and extradiol (such as C23O) cleaving enzymes (Cai et al. 2007). The genes of ring cleavage dioxygenases may serve as good targets for monitoring the biodegrading biomass, and also provide a rapid method for monitoring the microbial community during the treatment process (Heinaru et al. 2005).

The use of enzymes in industrial processes is usually linked to a reduced consumption of energy as well as chemicals and thus beneficial for the environment (Demarche et al. 2012). In biodegradation processes, free enzymes often undergo deactivation as a result of sudden changes in the surrounding environment, such as pH, temperature, and chemical and hydrodynamic shocks. These faults can be overcome by immobilization. Enzyme immobilization could enhance its stability against sudden changes in environmental factors: extend lifetime, increase reusability and easy handling. A few studies for the removal of phenolic pollutants by enzymatic treatment have been reported in recent years (Demarche et al. 2012; Fodil et al. 2012; Mohidem and Mat 2012; Lee et al. 2013). These studies have focused on offering novel, practical and inexpensive immobilization methods, utilizing various enzymes, such as laccases, peroxidases, tyrosinases and dioxygenases.

11 Future Prospective

In spite of the considerable amount of research on the biodegradation of CP’s, there is still a need for more effective approaches for theoretical and experimental investigations.The pathways and the degradation mechanisms should be evaluated through mathematical modeling to predict the effect of different factors on the degradation process. In recent years, researchers have mainly focused on the isolation and activities of the CPs-degrading bacteria in liquid media, and hence most of the reported findings are mainly limited to laboratory scale experiments. However, the results observed in the laboratory often differ from those observed in the field or in treatment processes in practice. Therefore, it is essential to carry out a large-scale direct study to evaluate the efficiency of CPs-degrading microbes in *in situ* conditions. Thus, innovative, effective and economical biodegradation methods should be proposed and evaluated at the laboratory scale and then applied to large scale to test their effectiveness.

12 Conclusion

The production and usage of large amounts of CPs have led to the entry of many contaminants into the environment. CPs are considered harmful to human health due to their potential carcinogenic and toxic effects. Although some of CPs are resistant to degradation and therefore persistent in the environment, microorganisms exposed to CPs have developed the ability to degrade some of them. Bacteria of different species have been reported to degrade CPs. Thus, such biological degradation can be exploited to remediate environmental pollution problems. In this chapter, some of the achievements in degrading of CPs by microorganisms have been reviewed. The degradation mechanism and pathways of two CPs; 2,4-dichlorophenol and Pentachlorophenol have been also discussed.

Different studies have revealed that CPs can be degraded aerobically or anaerobically, depending on the microbial abilities to grow in the particular environment. Aerobic microorganisms are more efficient for degrading CPs, because they grow fast and achieve complete mineralization of toxic organic compounds to inorganic compounds i.e. CO_2 and H_2O. For these reasons, there is a limited interest in the utilization of anaerobic bacteria for the degradation of CPs. Both pure and mixed cultures of the microorganisms have been utilized for the degradation of CP. *Pseudomonas putida* is one which is commonly used for the degradation due to their high removal efficiency. In fact, *P. putida* cells have been known to use diverse protective mechanisms for survival in various extreme environments.

Degradation rates are affected by a wide range of environmental factors which affect microbial action. For degradation to continue, bacteria, or their enzymes must be in contact with the CPs, and the CPs or its metabolites must be transported into the cell. Some bacteria display chemical responses to substrates, which may play an

important role in the environmental remediation. The environmental factors that regulate the degradation process include appropriate microorganisms, contact between the microbe and the substrate, pH, temperature, salinity, available water, oxygen, redox potential, nutrient availability, presence of alternative carbon substrates, light quality and intensity, binding to surfaces and alternative electron acceptor. In addition, molecular factors such as chemical structure, molecular weight and functional groups concentration and toxicity, and solubility in water can play a key role in the degradation process.

Acknowledgments The authors would like to thank Israa Elkonaissi and Riham Surkatti for their help in preparing this chapter.

References

Abrahamsson, K., & Klick, S. (1991). Degradation of halogenated phenols in anoxic marine sediments. *Marine Pollution Bulletin, 22*, 227–233.

Agarry, S. E., Durojaiye, A. O., & Solomon, B. O. (2008). Microbial degradation of phenols: A review. *International Journal of Environment and Pollution, 32*, 12–28.

Aislabie, J., & Lloyd-Jones, G. (1995). A review of bacterial-degradation of pesticides. *Australian Journal of Soil Research, 33*, 925–942.

Al-Khalid, T., & El-Naas, M. (2013). Transient behavior in biodegradation of 2, 4 dichlorophenol: Is it a starvation effect? *International Journal of Chemical Engineering and Applications, 4*, 365–368.

Al-Khalid, T., & El-Naas, M. (2014). Biodegradation of phenol and 2, 4 dichlorophenol: The role of glucose in biomass acclimatization. *International Journal of Engineering Research and Technology, 3*, 1579–1586.

Al-Khalid, T., & El-Naas, M. H. (2011). Aerobic biodegradation of phenols: A comprehensive review. *Critical Reviews in Environmental Science and Technology, 42*, 1631–1690.

Al-Thani, R. F., Abd-El-Haleem, A. M., & Al-Shammri, A. (2007). Isolation, biochemical and molecular characterization of 2-chlorophenol-degrading Bacillus isolates. *African Journal of Biotechnology, 6*, 2675–2681.

Al-Zuhair, S., & El-Naas, M. H. (2012). Phenol biodegradation by *Ralstonia pickettii* extracted from petroleum refinery oil sludge. *Chemical Engineering Communications, 199*, 1194–1204.

Alva, V., & Peyton, B. (2003). Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: Influence of pH and salinity. *Environmental Science and Technology, 37*, 4397–4402.

Alvarez-Cohen, L., & McCarty, P. L. (1991). A cometabolic biotransformation model for halogenated aliphatic compounds exhibiting product toxicity. *Environmental Science and Technology, 25*, 1381–1387.

Alvarez-Cohen, L., & Speitel, J. (2001). Kinetics of aerobic cometabolism of chlorinated solvents. *Biodegradation, 12*, 105–126.

An, H.-R., Park, H.-J., & Kim, E.-S. (2001). Cloning and expression of thermophilic catechol 1,2-dioxygenase gene (catA) from *Streptomyces setonii*. *FEMS Microbiology Letters, 195*, 17–22.

Annachhatre, A. P., & Gheewala, S. H. (1996). Biodegradation of chlorinated phenolic compounds. *Biotechnology Advances, 14*, 35–56.

Antizar-Ladislao, B., & Galil, N. (2004). Absorption of phenol and chlorophenols by acclimated residential biomass under bioremediation conditions in study aquifer. *Water Research, 38*, 267–276.

Apajalahti, J. H. A., & Salkinoja-Salonen, M. S. (1987). Complete dechlorination of tetrachlorohydroquinone by cell-extracts of pentachlorophenol-induced *Rhodococcus chlorophenolicus*. *Journal of Bacteriology, 169*, 5125–5130.

Araya, M. & Lakhi, A. (2004). Response to consecutive nematicide applications using the same product in mussa AAAcv. *Grande naine* originated from *in vitro* propagative material and cultivated in virgin soil. *Nematologia Brasileira 28,* 55.

Armenante, P. M., Kafkewitz, D., Lewandowski, G. A., & Jou, C.-J. (1999). Anaerobic–Aerobic treatment of halogenated phenolic compounds. *Water Research, 33*, 681–692.

Arora, P., & Bae, H. (2014). Bacterial degradation of chlorophenols and their derivatives. *Microbial Cell Factories, 13*, 31.

ATSDR (1999). Toxicological profile for chlorophenols. In Services, U.D. o. H.a.H. (ed.). Atlanta, GA.

Azbar, N., Tutuk, F., & Keskin, T. (2009). Biodegradation performance of an anaerobic hybrid reactor treating olive mill effluent under various organic loading rates. *International Biodeterioration and Biodegradation, 63*, 690–698.

Bae, H. S., Rhee, S. K., Cho, Y. G., Hong, J. K., & Lee, S. T. (1997). Two different pathways (a chlorocatechol and a hydroquinone pathway) for the 4-chlorophenol degradation in two isolated bacterial strains. *Journal of Microbiology and Biotechnology, 7*, 237–241.

Bajaj, M., Gallert, C., & Winter, J. (2009). Treatment of phenolic wastewater in an anaerobic fixed bed reactor (AFBR)—Recovery after shock loading. *Journal of Hazardous Materials, 162*, 1330–1339.

Baker, M. D., & Mayfield, C. J. (1980). Microbial and non-biological decomposition of chiorophenols and phenol in soil. *Water, Air, and Soil Pollution, 13*, 411–424.

Basu, S. K., Oleszkiewicz, J. A., & Sparling, R. (2005). Effect of sulfidogenic and methanogenic inhibitors on reductive dehalogenation of 2-chlorophenol. *Environmental Technology, 26*, 1383–1391.

Becker, J. G., Stahl, D. A., & Rittmann, B. E. (1999). Reductive dehalogenation and conversion of 2-chlorophenol to 3- chlorobenzoate in a methanogenic sediment community: Implications for predicting the of chlorinated pollutants. *Applied and Environment Microbiology, 65*, 5169–5172.

Bergauer, P., Fonteyne, P. A., Nolard, N., Schinner, F., & Margesin, R. (2005). Biodegradation of phenol and phenol-related compounds by psychrophilicand cold-tolerant alpine yeasts. *Chemosphere, 59*, 909–918.

Boothe, D. D. H., Rogers, J. E., & Wiegel, J. (1997). Reductive dechlorination of chlorophenols in slurries of low- organic-carbon marine sediments and subsurface soils. *Applied Microbiology and Biotechnology, 47*, 742–748.

Boyd, S. A., & Shelton, D. R. (1984). Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. *Applied and Environment Microbiology, 47*, 272–277.

Breining, S., Schilts, E., & Fuchs, G. (2000). Genes involved in metabolism of phenol in bacterium *Thauera aromatica*. *Journal of Bacteriology, 182*, 5849–5863.

Brown, J. F., Bedard, D. L., Brennan, M. J., Camahan, J. C., Feng, H., & Wagner, R. E. (1987). Polychlorinated biphenyl dechlorination in aquatic sediments. *Science, 236*, 709–712.

Cai, W., Li, J., & Zhang, Z. (2007). The characteristics and mechanisms of phenol biodegradation by *Fusarium* sp. *Journal of Hazardous Materials, 148*, 38–42.

Caliz, J., Vila, X., Martí, E., Sierra, J., Nordgren, J., Lindgren, P.-E., et al. (2011). The microbiota of an unpolluted calcareous soil faces up chlorophenols: Evidences of resistant strains with potential for bioremediation. *Chemosphere, 83*, 104–116.

Cea, M., Seaman, J. C., Jara, A., Fuentes, B., Mora, M. L., & Diez, M. C. (2007). Adsorption behaviour of 2,4-dichlorophenol and pentachlorophenol in an allophanic soil. *Chemosphere, 67*, 1354–1360.

Christen, P., Vega, A., Casalot, L., Simon, G., & Auria, R. (2012). Kinetics of aerobic phenol biodegradation by the acidophilic and hyperthermophilic archaeon *Sulfolobus solfataricus* 98/2. *Biochemical Engineering Journal, 62*, 56–61.

Copley, S. D. (1997). Diverse mechanistic approaches to difficult chemical transformations: Microbial dehalogenation of chlorinated aromatic compounds. *Chemistry and Biology, 4*, 169–174.

Cortes, D., Garrios-Gonzalez, J., & Tomasini, A. (2002). Pentachlorophenol tolerance and removal by *Rhizopus nigricans* in solid-state culture. *Process Biochemistry, 37*, 881–884.

Crawford, R. L., Jung, C. M., & Strap, J. L. (2007). The recent evolution ofpentachlorophenol (PCP)-4-monooxygenase (PcpB) and associated pathways for bacterial degradation of PCP. *Biodegradation, 18*, 525–539.

Czaplicka, M. (2004). Sources and transformations of chlorophenols in the natural environment. *Science of the Total Environment, 322*, 21–39.

D'Angelo, E. M., & Reddy, K. R. (2000). Aerobic and anaerobic transformations of pentachlorophenol in wetland soils. *Soil Science Society of America Journal, 64*, 933–943.

Dapaah, S. Y., & Hill, G. A. (1992). Biodegradation of chlorophenol mixtures by *Pseudomonas putida*. *Biotechnology and Bioengineering, 40*(11), 1353–1358.

Demarche, P., Junghanns, C., Nair, R. R., & Agathos, S. N. (2012). Harnessing the power of enzymes for environmental stewardship. *Biotechnology Advances, 30*, 933–953.

Demnerova, K., Mackova, M., Spevakova, V., Beranova, K., Kochankova, L., Lovecka, P., et al. (2005). Two approaches to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial populations. *International Microbiology, 8*, 205–211.

Diez, M. C., Gallardo, F., Tortella, G., Rubilar, O., Navia, R., & Bornhardt, C. (2012). Chlorophenol degradation in soil columns inoculated with Anthracophyllum discolor immobilized on wheat grains. *Journal of Environmental Management, 95*(Suppl), S83–S87.

Diez, M. C., Mora, M. L., & Videla, S. (1999). Adsorption of phenolic compounds and color from bleached Kraft mill effluent using allophanic compounds. *Water Research, 33*, 125–130.

DiVincenzo, J., & Sparks, D. L. (2001). Sorption of the neutral and charges forms of pentachlorophenol on soil: evidence for different mechanisms. *Archives of Environmental Contamination and Toxicology, 40*, 445–450.

Dong, X., Hong, Q., He, L., Jiang, X., & Li, S. (2009). Characterization of phenol-degrading bacterial strains isolated from natural soil. *International Biodeterioration and Biodegradation, 63*, 365–370.

El-Naas, M. H., Al-Muhtaseb, S. A., & Makhlouf, S. (2009). Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *Journal of Hazardous Materials, 164*, 720–725.

El-Naas, M. H., Al-Zuhair, S., & Makhlouf, S. (2010). Continuous biodegradation of phenol in a spouted bed bioreactor (SBBR). *Chemical Engineering Journal, 160*, 565–570.

Esteve-Núñez, A., Caballero, A., & Ramos, J. L. (2001). Biological degradation of 2,4,6-rinitrotoluene. *Microbiology and Molecular Biology Reviews, 65*, 335–352.

Farrell, A., & Quilty, B. (2002). The enhancement of 2-chlorophenol degradation by a mixed microbial community when augmented with *Pseudomonas putida* CP1. *Water Research, 36*, 2443–2450.

Fava, F., Armenante, P. M., & Kafkewitz, D. (1995). Aerobic degradation and dechlorination of 2 = chlorophenol, 3-chlorophenol and 4-chlorophenol by a *Pseudomonas pickettii* strain. *Letters in Applied Microbiology, 21*, 307–312.

Ferraroni, M., Kolomytseva, M. P., Solyanikova, I. P., Scozzafava, A., Golovleva, L. A., & Briganti, F. (2006). Crystal structure of 3-chlorocatechol 1,2-dioxygenase key enzyme of a new modified ortho-pathway from the gram-positive *Rhodococcus opacus* 1CP grown on 2-chlorophenol. *Journal of Molecular Biology, 360*, 788–799.

Fewson, C. A. (1988). Biodegradation of xenobiotic and other persistent compounds: The causes of recalcitrance. *Trends in Biotechnology, 6*, 148–153.

Field, J. A., & Sierra-Alvarez, R. (2008). Microbial transformation and degradation of polychlorinated biphenyls. *Environmental Pollution, 155*, 1–12.

Finkel'shtein, Z. I., Baskunov, B. P., Golovlev, E. L., Moiseeva, O. V., Vervoort, J., Rietjens, I., et al. (2000). Dependence of the conversion of chlorophenols by rhodococci on the number and position of chlorine atoms in the aromatic ring. *Microbiology, 69*, 40–70.

Fodil, D., Jaouadi, B., Badis, A., Nadia, Z. J., Ferradji, F. Z., Bejar, S., et al. (2012). A thermostable humic acid peroxidase from *Streptomyces* sp. strain AH4: Purification and biochemical characterization. *Bioresource Technology, 111*, 383–390.

Gallizia, I., McClean, S., & Banat, I. (2003). Bacterial biodegradation of phenol and 2,4-dichlorophenol. *Journal of Chemical Technology and Biotechnology, 78*, 959–963.

Gaofeng, W., Hong, X., & Mei, J. (2004). Biodegradation of chlorophenols: A review. *Chemical Journal on Internet, 6*, 1–67.

Gibson, S. A., & Suflita, J. M. (1986). Extrapolation of biodegradation results to groundwater aquifers: Reductive dehalogenation of aromatic compounds. *Applied and Environment Microbiology, 52*, 681–688.

Haggblom, M. M., Apajalahti, J. H., & Salkinoja-Salonen, M. S. (1988a). O-methylation of chlorinated para-hydroquinones by *Rhodococcus chlorophenolicus*. *Applied and Environment Microbiology, 54*, 1818–1824.

Haggblom, M. M., Nohynek, L. J., & Salkinoja Salonen, M. S. (1988b). Degradation and o-methylation of chlorinated phenolic-compounds by *Rhodococcus* and *Mycobacterium* strains. *Applied and Environment Microbiology, 54*, 3043–3052.

Haggblom, M. M., Rivera, M. D., & Young, L. Y. (1993). Influence of alternative electron-acceptors on the anaerobic biodegradability of chlorinated phenols and benzoic-acids. *Applied and Environment Microbiology, 59*, 1162–1167.

Hahn, D., Cozzolino, A., Piccolo, A., & Armenante, P. M. (2007). Reduction of 2,4-dichlorophenol toxicity to Pseudomonas putida after oxidative incubation with humic substances and a biomimetic catalyst. *Ecotoxicol Environ Safety, 66*, 335–342.

Hale, D. D., Reineke, W., & Wiegel, J. (1994). Chlorophenol degradation. In G. R. Chaudhry (Ed.), *Biological degradation and bioremediation of toxic chemicals* (pp. 74–91). London, UK: Chapman and Hall.

Hao, O. J., Kim, M. H., Seagren, E. A., & Kim, H. (2002). Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species. *Chemosphere, 46*, 797–807.

Heinaru, E., Merimaa, M., Viggor, S., Lehiste, M., Leito, I., Truu, J., et al. (2005). Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. *FEMS Microbiology Ecology, 51*, 363–373.

Horowitz, A., Suflita, J. M., & Tiedje, J. M. (1983). Reductive dehalogenation of halobenzoates by anaerobic lake sediment microorganisms. *Applied and Environment Microbiology, 45*, 1459–1465.

Horvath, R. S. (1972). Microbial co-metabolism and the degradation of organic compounds in nature. *Bacteriological Reviews, 36*, 146–155.

Hsieh, F.-M., Huang, C., Lin, T.-F., Chen, Y.-M., & Lin, J.-C. (2008). Study of sodium tripolyphosphate-crosslinked chitosan beads entrapped with *Pseudomonas putida* for phenol degradation. *Process Biochemistry, 43*, 83–92.

Hussain, A., Kumar, P., & Mehrotra, I. (2010). Nitrogen biotransformation in anaerobic treatment of phenolic wastewater. *Desalination, 250*, 35–41.

Hwang, H. M., Hodson, R. E., & Lee, R. F. (1986). Degradation of phenol and chlorophenols by sunlight and microbes in estuarine water. *Environmental Science and Technology, 20*, 1002–1007.

Iranzo, M., Sain-Pardo, I., Boluda, R., Sanchez, J., & Mor-meneo, S. (2001). The use of microorganisms in envi-ronmental remediation. *Annals of Microbiology, 51*, 135–143.

Ivanciuc, T., Ivanciuc, O., & Douglas, J. K. (2006). Prediction of environmental properties for chlorophenols with posetic quantitative super-structure/property relationships (QSSPR). *International Journal of Molecular Sciences, 7*, 358–374.

Janke, D., Ihn, W., & Tresselt, D. (1989). Critical steps in degradation of chloroaromatics by *Rhodococci*. IV. Detailed kinetics of substrate removal and product formation by resting preadapted cells. *Journal of Basic Microbiology, 29*, 1–10.

Jensen, J. (1996). Chlorophenols in the terrestrial environment. *Reviews of Environmental Contamination and Toxicology, 146*, 211–223.

Jiang, H.-L., Tay, T.-L., Maszenan, A. M., & Tay, J.-H. (2006). Physiological traits of bacterial strains isolated from phenol-degrading aerobic granules. *FEMS Microbiology Ecology, 57*, 182–191.

Jones, P. A. (1983). *Chlorophenols and their impurities in the Canadian environment*. Environmental Protection Service: Environment Canada.

Kargi, F., & Eker, S. (2004). Toxicity and batch biodegradation kinetics of 2,4 dichlorophenol by pure *Pseudomonas putida* culture. *Enyzme and Microbial Technology, 35*, 424–428.

Kim, J.-H., Oh, K.-K., Lee, S.-T., Kim, S.-W., & Hong, S.-I. (2002). Biodegradation of phenol and chlorophenols with defined mixed culture in shake-flasks and a packed bed reactor. *Process Biochemistry, 37*, 1367–1373.

Kim, M. H., & Hao, O. J. (1999). Cometabolic degradation of chlorophenols by *Acinetobacter* species. *Water Research, 33*, 562–574.

Kiyohara, H., Takizawa, N., Uchiyama, T., Ikarugi, H., & Nagao, K. (1989). Degradability of polychlorinated phenols by bacterial populations in soil. *Journal of Fermentation and Bioengineering, 67*, 339–344.

Klekner, V., & Kosaric, N. (1992). Degradation of phenolic mixtures by *chlorella*. *Environmental Technology, 13*, 503–506.

Kohring, G. W., Rogers, J. E., & Wiegel, J. (1989). Anaerobic biodegradation of 2,4-dichlorophenol in fresh-water lake-sediments at different temperatures. *Applied and Environment Microbiology, 55*, 348–353.

Kookana, R. S., & Rogers, S. L. (1995). Effects of pulp mill effluent disposal on soil. *Reviews of Environmental Contamination and Toxicology, 142*, 13–64.

Krieg, N. R., & Holt, J. C. (Eds.). (1984). *Bergey's Manual of Systematic Bacteriology* (1st ed., Vol. 1). Baltimore: Williams and Wilkins.

Laine, M. M., & Jørgensen, K. S. (1997). Effective and safe composting of chlorophenol-contaminated soil in pilot scale. *Environmental Science and Technology, 31*, 371–378.

Lallai, A., & Mura, G. (2004a). Biodegradation of 2-chlorophenol in forest soil: effect of inoculation with aerobic sewage sludge. *Environmental Toxicology and Chemistry, 23*, 325–330.

Lallai, A., & Mura, G. (2004b). Biodegradation of 2-chlorophenol in forest soil: effect ofinoculation with aerobic sewage sludge. *Environmental Toxicology and Chemistry, 23*(2), 325–330.

Larsson, P., & Lemkemeier, K. (1989). Microbial mineralization of chlorinated phenols and biphenyls in sediment-water systems from humic and clear-water lakes. *Water Research, 23*, 1081–1085.

Lee, S. H., Lee, S. H., Ryu, S. J., Kang, C. S., Suma, Y., & Kim, H. S. (2013). Effective biochemical decomposition of chlorinated aromatic hydrocarbons with a biocatalyst immobilized on a natural enzyme support. *Bioresource Technology, 141*, 89–96.

Levén, L., Nyberg, K., Korkea-aho, L., & Schnürer, A. (2006). Phenols in anaerobic digestion processes and inhibition of ammonia oxidising bacteria (AOB) in soil. *Science of the Total Environment, 364*, 229–238.

Li, J., Cai, W., & Zhu, L. (2011). The characteristics and enzyme activities of 4-chlorophenol biodegradation by *Fusarium* sp. *Bioresource Technology, 102*, 2985–2989.

Li, Y., Li, J., Wang, C., & Wang, P. (2010). Growth kinetics and phenol biodegradation of psychrotrophic *Pseudomonas putida* LY1. *Bioresource Technology, 101*, 6740–6744.

Lika, K., & Papadakis, I. A. (2009). Modeling the biodegradation of phenolic compounds by microalgae. *Journal of Sea Research, 62*, 135–146.

Liu, S.-M., Kuo, C.-E., & Hsu, T.-B. (1996). Reductive dechlorination of chlorophenols and pentachlorophenol in anoxic estuarine sediments. *Chemosphere, 32*, 1287–1300.

Loh, K.-C., & Cao, B. (2008). Paradigm in biodegradation using *Pseudomonas putida*—A review of proteomics studies. *Enyzme and Microbial Technology, 43*, 1–12.

Loh, K.-C., & Wang, S.-J. (1998). Enhancement of biodegradation of phenol and a non-growth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation, 8*, 329–338.

Londry, K. L., & Fedorak, P. M. (1992). Benzoic acid intermediates in the anaerobic biodegradation of phenols. *Canadian Journal of Microbiology, 38*, 1–11.

Lu, C.-J., Lee, C.-M., & Huang, C.-Z. (1996). Biodegradation of chlorophenols by immobilized pure-culture microorganisms. *Water Science and Technology, 34*, 67–72.

Mahmood, S., Paton, G. I., & Prosser, J. I. (2005). Cultivation-independent in situ molecular analysis of bacteria involved in degradation of pentachlorophenol in soil. *Environmental Microbiology, 7*, 1349–1360.

Majumder, P. S., & Gupta, S. K. (2007). Removal of chlorophenols in sequential anaerobic–aerobic reactors. *Bioresource Technology, 98*, 118–129.

Majumder, P. S., & Gupta, S. K. (2009). Effect of influent pH and alkalinity on the removal of chlorophenols in sequential anaerobic–aerobic reactors. *Bioresource Technology, 100*, 1881–1883.

Margesin, R., Moertelmaier, C., & Mair, J. (2013). Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene,) by four actinobacterial strains. *International Biodeterioration and Biodegradation, 84*, 185–191.

Martinez, A. T., Speranza, M., Ruiz-Duenas, F. J., Ferreira, P., Camarero, S., Guillen, F., et al. (2005). Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology, 8*, 195–204.

Matus, V., Sanchez, M. A., Martinez, M., & Gonzalez, B. (2003). Efficient degradation of 2,4,6-trichlorophenol requires a set of catabolic genes related to tcp genes from *Ralstonia eutropha* JMP134 (pJP4). *Applied and Environment Microbiology, 69*, 7108–7115.

Menke, B., & Rehm, H.-J. (1992). Degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes* sp. A 7–2. *Applied Microbiology and Biotechnology, 37*, 655–661.

Michałowicz, J., & Duda, W. (2007). Phenols—sources and toxicity. *Polish Journal of Environmental Studies, 16*, 347–362.

Middeldorp, P., De Wolf, J., Zehnder, A., & Schraa, G. (1997). Enrichment and properties of a 1,2,4-trichlorobenzene-dechlorinating methanogenic microbial consortium. *Applied and Environment Microbiology, 63*, 1225–1229.

Mikesell, M. D., & Boyd, S. A. (1986). Complete reductive dechlorination and mineralization of pentachlorophenol by anaerobic microorganisms. *Applied and Environment Microbiology, 52*, 861–865.

Milliken, C. E., Meier, G. P., Sowers, K. R., & May, H. D. (2004). Chlorophenol production by anaerobic microorganisms: Transformation of a biogenic chlorinated hydroquinone metabolite. *Applied and Environment Microbiology, 70*, 2494–2496.

Mohidem, N. A., & Mat, H. B. (2012). The catalytic activity enhancement and biodegradation potential of free laccase and novel sol–gel laccase in non-conventional solvents. *Bioresource technology, 114*, 472–477.

Mohn, W. M., & Kennedy, K. J. (1992). Reductive dehalogination of chlorophenols by *Desulfomonile tiedje* DCB-1. *Applied and Environment Microbiology, 58*, 1367–1370.

Moorman, T. B., Cowan, J. K., Arthur, E. L., & Coats, J. R. (2001). Organic amendments to enhance herbicide biodegradation in contaminated soils. *Biology and Fertility of Soils, 33*, 541–545.

Murialdo, S. E., Fenoglio, R., Haure, P. M., & González, J. F. (2003). Degradation of phenol and chlorophenols by mixed and pure cultures. *Water SA, 29*, 457–463.

Muñoz, R., Souza, T. S. O., Glittmann, L., Pérez, R., & Quijano, G. (2013). Biological anoxic treatment of O_2-free VOC emissions from the petrochemical industry: A proof of concept study. *Journal of Hazardous Materials, 260*, 442–450.

Namkoong, W., Loehr, R. C., & Malina, J. F. (1988). Kinetics of phenolic compoundsremoval in soil. *Hazardous Waste and Hazardous Materials, 5*, 321–328.

Nordin, K., Unell, M., & Jansson, J. K. (2005). Novel 4-chlorophenol degradation gene cluster and degradation route via hydroxyquinol in *Arthrobacter chlorophenolicus* A6. *Applied and Environment Microbiology, 71*, 6538–6544.

Nuhoglu, A., & Yalcin, B. (2005). Modelling of phenol removal in a batch reactor. *Process Biochemistry, 40*, 1233–1239.

Oberg, L. G., & Rappe, C. (1992). Biochemical formation of PCDD/Fs from chlorophenols. *Chemosphere, 25*, 49–52.

Okpokwasili, G. C., & Nweke, C. O. (2005). Microbial growth and substrate utilization kinetics. *African Journal of Biotechnology, 5*, 305–307.

Olaniran, A. O., & Igbinosa, E. O. (2011). Chlorophenols and other related derivatives of environmental concern: Properties, distribution and microbial degradation processes. *Chemosphere, 83*, 1297–1306.

Onysko, K. A., Budman, H. M., & Robinson, C. W. (2000). Effect of temperature on the inhibition kinetics of phenol biodegradation by *Pseudomonas putida* Q5. *Biotechnology and Bioengineering, 70*, 291–299.

Orser, C. S., & Lange, C. C. (1994). Molecular analysis of pentachlorophenol degradation. *Biodegradation, 5*, 277–288.

Palleroni, N. J. (1986). Taxonomy of the *Pseudomonads*. In J. R. Sokatch (Ed.), *The bacteria* (Vol. X, pp. 3–25). The biology of *Pseudomonads*. New York: Academic Press.

Pandey, G., Paul, D., & Jain, R. K. (2003). Branching of o-nitrobenzoate degradation pathway in *Arthrobacter protophormiae* RKJ100: Identification of new intermediates. *FEMS Microbiology Letters, 229*, 231–236.

Papazi, A., & Kotzabasis, K. (2007). Bioenergetic strategy of microalgae for the biodegradation of phenolic compounds—Exogenously supplied energy and carbon sources adjust the level of biodegradation. *Journal of Biotechnology, 129*, 706–716.

Park, H.-J., & Kim, E.-S. (2003). An inducible Streptomyces gene cluster involved in aromatic compound metabolism. *FEMS Microbiology Letters, 226*, 151–157.

Pedroza, A. M., Mosqueda, R., Alonso-Vante, N., & Rodríguez-Vázquez, R. (2007). Sequential treatment via and UV//RuxSey to reduce contaminants in waste water resulting from the bleaching process during paper production. *Chemosphere, 67*, 793–801.

Pieper, D., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology, 11*, 262–270.

Pignatello, J. J., Martinson, M. M., Steiert, J. G., Carlson, R. E., & Crawford, R. L. (1983). Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. *Applied and Environment Microbiology, 46*(5), 1024–1031.

Potgieter, J. H., Bada, S. O., & Potgieter-Vermaak, S. S. (2009). Adsorption removal of various phenol from water by South African coal fly ash. *Water SA, 35*, 110–112.

Puyol, D., Mohedano, A. F., Rodriguez, J. J., & Sanz, J. L. (2011). Effect of 2,4,6-trichlorophenol on the microbial activity of adapted anaerobic granular sludge bioaugmented with *Desulfito bacterium* strains. *New Biotechnology, 29*, 79–89.

Qiu, X., Zhong, Q., Li, M., Bai, W., & Li, B. (2007). Biodegradation of p-nitrophenol by methyl parathion-degrading *Ochrobactrum* sp. B2. *International Biodeterioration and Biodegradation, 59*, 297–301.

Quan, X., Shi, H., Zhang, Y., Wang, J., & Qian, Y. (2004). Biodegradation of 2,4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *Achromobacter* sp. *Separation and Purification Technology, 34*, 97–103.

Reddy, G. V. B., & Gold, M. H. (2000). Degradation of pentachlorophenol by *Phanerochaete chrysosporium*: Intermediates and reactions involved. *Microbiology, 146*, 405–413.

Ren, M., Song, Y., Xiao, S., Zeng, P., & Peng, J. (2011). Treatment of berberine hydrochloride wastewater by using pulse electro-coagulation process with Fe electrode. *Chemical Engineering Journal, 169*, 84–90.

Rubilar, O., Diez, M. C., & Gianfreda, L. (2008). Transformation of chlorinated phenolic compounds by white rot fungi. *Critical Reviews in Environmental Science and Technology, 38*, 227–268.

Ryan, M. P., Pembroke, J. T., & Adley, C. C. (2007). *Ralstonia pickettii* in environmental biotechnology: Potential and applications. *Journal of Applied Microbiology, 103*, 754–764.

Sahinkaya, E., & Dilek, F. B. (2006a). Biodegradation of 4-CP and 2,4-DCP mixture in a rotating biological contactor (RBC). *Biochemical Engineering Journal, 31*, 141–147.

Sahinkaya, E., & Dilek, F. B. (2006b). Effect of biogenic substrate concentration on the performance of sequencing batch reactor treating 4-CP and 2,4-DCP mixtures. *Journal of Hazardous Materials, 128*, 258–264.

Sahinkaya, E., & Dilek, F. B. (2007). Biodegradation kinetics of 2,4-dichlorophenol by acclimated mixed cultures. *Journal of Biotechnology, 127*, 716–726.

Sanchez, M. A., Vasquez, M., & Gonzalez, B. (2004). A previously unexposed forest soil microbial community degrades high levels of the pollutant 2,4,6-trichlorophenol. *Applied and Environment Microbiology, 70*, 7567–7570.

Scelza, R., Rao, M. A., & Gianfreda, L. (2008). Response of an agricultural soil to pentachlorophenol (PCP) contamination and the addition of compost or dissolved organic matter. *Soil Biology and Biochemistry, 40*, 2162–2169.

Semprini, L. (1997). Strategies for the aerobic co-metabolism of chlorinated solvents. *Current Opinion in Biotechnology, 8*, 296–308.

Sharpee, K. W., Duxbury, J. M., & Alexande, M. (1973). 2,4-Dichlorophenoxyacetate metabolism by *Arthrobacter* sp.: Accumulation of a chlorobutenolide. *Journal of Applied Microbiology, 26*, 445–447.

Shourian, M., Noghabi, K. A., Zahiri, H. S., Bagheri, T., Karballaei, G., Mollaei, M., et al. (2009). Efficient phenol degradation by a newly characterized *Pseudomonas* sp. SA01 isolated from pharmaceutical wastewaters. *Desalination, 246*, 577–594.

Singh, B., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *Microbiology Reviews, 30*, 428–471.

Sittig, M. (1981). Handbook of toxic and hazardous chemicals.

Siuda, J. F. (1980). Natural production of organohalogens. In: *Water chlorination: Environmental impact and health effects*, vol 3 (pp. 63–72). Science Publishers Inc.

Smith, J. A., & Novak, J. T. (1987). Biodegradation of chlorinated phenols in subsurface soils. *Water, Air, and Soil Pollution, 33*, 29–42.

Solyanikova, I. P., & Golovleva, L. A. (2004). Bacterial degradation of chlorophenols: Pathways, biochemica and genetic aspects. *Journal of Environmental Science and Health, B39*, 333–351.

Stehlickova, L., Svab, M., Wimmerova, L., & Kozler, J. (2009). Intensification of phenol biodegradation by humic substances. *International Biodeterioration and Biodegradation, 63*, 923–927.

Steinle, P., Stucki, G., Stettler, R., & Hanselmann, W. (1998). Aerobic mineralization of 2,6-Dichlorophenol by *Ralstonia* sp. strain RK1. *Applied and Environment Microbiology, 64*, 2566–2571.

Stoilova, I., Krastanov, A., Stanchev, V., Daniel, D., Gerginova, M., & Alexieva, Z. (2006). Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. *Enyzme and Microbial Technology, 39*, 1036–1041.

Stoilova, I., Krastanov, A., Yanakieva, I., Kratchanova, M., & Yemendjiev, H. (2007). Biodegradation of mixed phenolic compounds by *Aspergillus awamori* NRRL 3112. *nternational Biodeterioration and Biodegradation, 60*, 342–346.

Susarla, S., Yonezawa, Y., Nakanishi, J., & Masunaga, S. (1997). Anaerobic transformation kinetics and pathways of chlorophenols in fresh water lake sediment. *Water Science and Technology, 36*, 99–105.

Suzuki, T. (1983). Methylation and hydroxylation of pentachlorophenol by mycobacterium-sp isolated from soil. *Journal of Pesticide Science, 8*, 419–428.

Takeuchi, R., Suwa, Y., Yamagishi, T., & Yonezawa, Y. (2000). Anaerobic transformation of chlorophenols in methanogenic sludge unexposed to chlorophenols. *Chemosphere, 41*, 1457–1462.

Tamer, E., Hamid, Z., Aly, A. M., Ossama, E. T., Bo, M., & Benoit, G. (2006). Sequential UV–biological degradation of chlorophenols. *Chemosphere, 63*, 277–284.

Tiedje, J. M., Duxbury, J. M., Alexande, M., & Dawson, J. E. (1969). 2,4-D metabolism: Pathway of degradation of chlorocatechols by *Arthrobacter* sp. *Journal of Agricultural and Food Chemistry, 17*, 1021–1026.

Tobajas, M., Monsalvo, V. M., Mohedano, A. F., & Rodriguez, J. J. (2012). Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by *Comamonas testosteroni*. *Journal of Environmental Management, 95*(Suppl), 116–121.

Uotila, J., Kitunen, V., Saastamoinen, T., Coote, T., Haggblom, M., & Salkinoja-Salonen, M. (1992). Characterization of aromatic dehalogenases of *Mycobacterium fortuitum* CG-2. *Journal of Bacteriology, 174*, 5669–5675.

Uotila, J. S., & Salkinoja-Salonen, M. S. (1991). Dechlorination of pentachlorophenol by membrane boundenzymes of *Rhodococcus chlorophenolicus* PCP-I. *Biodegradation, 2*, 25–31.

Valo, R. J., & Salkinoja-Salonen, M. S. (1986). Bioreclamation of chlorophenol-contaminated soilby composting. *Applied Microbiology and Biotechnology, 25*, 68–75.

Van Agteren, M. H., Keuning, S., & Janssen, D. B. (1998). *Handbook on biodegradation and biological treatment of hazardous organic compounds*. London: Kluwer Academic Publishers.

Vischetti, C., Casucci, C., Perucci, P. (2002). *Relationship between changes of soil microbial biomass content and imazamox and benfluralin degradation*. Springer Verlag.

Wang, S. J. (1997). Influence of conventional carbon supplements on biodegradation of phenol and cometabolite, 4-chlorophenol (4-CP). ME thesis, National University of Singapore.

Wang, S.-G., Liu, X.-W., Zhang, H.-Y., Gong, W.-X., Sun, X.-F., & Gao, B.-Y. (2007a). Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor. *Chemosphere, 69*, 769–775.

Wang, Y., Tian, Y., Han, B., H-b, Zhao, J-n, Bi, & B-l, Cai. (2007b). Biodegradation of phenol by free and immobilized *Acinetobacter* sp. strain PD12. *Journal of Environmental Science, 19*, 222–225.

Warner, K. A., Gilmour, C. C., & Capone, D. G. (2002). Reductive dechlorination of 2,4-dichlorophenol and related microbial processes under limiting and non-limiting sulfate concentration in anaerobic mid-Chesapeake Bay sediments. *FEMS Microbiology Ecology, 40*, 159–165.

Watanabe, I. (1978). Pentachlorophenol (PCP) decomposing activity of field soils treated annually with PCP. *Soil Biology and Biochemistry, 10*, 71–75.

Weber, R., Gaus, C., & Tysklind, M. (2008). Dioxin- and POP-contaminated sites—contemporary and future relevance and challenges: Overview on background aims and scope of the series. *Environmental Science and Pollution Research, 15*, 363–393.

Xun, L. Y., & Webster, C. M. (2004). A monooxygenase catalyzes sequential dechlorinations of 2,4,6-trichlorophenol by oxidative and hydrolytic reactions. *Journal of Biological Chemistry, 279*, 6696–6700.

Yang, R. D., & Humphrey, A. E. (1975). Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. *Biotechnology and Bioengineering, 17*, 1211–1235.

Yang, S., Shibata, A., Yoshida, N., & Katayama, A. (2009). Anaerobic mineralization of pentachlorophenol (PCP) by combining PCP-dechlorinating and phenol-degrading cultures. *Biotechnology and Bioengineering, 102*, 81–90.

Yu, Z., Zeng, G.-M., Chen, Y.-N., Zhang, J.-C., Yu, Y., Li, H., et al. (2011). Effects of inoculation with Phanerochaete chrysosporium on remediation of pentachlorophenol-contaminated soil waste by composting. *Process Biochemistry, 46*, 1285–1291.

Zhang, C., & Bennett, G. N. (2005). Biodegradation of xenobiotic by anaerobic bacteria. *Applied Microbiology and Biotechnology, 67*(5), 600–618.

Zhang, G., Gao, Y., Zhang, Y., & Gu, P. (2005). Removal of fluoride from drinking water by a membrane coagulation reactor (MCR). *Desalination, 177*, 143–155.

Zhang, X., & Wiegel, J. (1990). Sequential anaerobic degradation of 2,4- dichlorophenol in freshwater sediments. *Applied and Environment Microbiology, 56*, 1119–1127.

Remediation of Endosulfan Contaminated System by Microbes

Mathava Kumar and Ligy Philip

1 Endosulfan: Characteristics, Usage and Environmental Issues

The use of agrochemicals to control the uncountable production loss due to pest attack is inevitable. Due to this reason, production of pesticides has increased many folds in recent years. Environmental contamination due to the excessive use of pesticides has become a great concern to the public and to the environmental regulatory authorities. Owing to their disastrous effects, biological pest controlling methods are gaining importance in recent years. Pesticides, starting from the synthesis of DDT (4-, 4′-Dichloro Diphenyl Trichloroethane) in 1874 to the recently developed pyrethroids, have undergone a tremendous rapid growth. However, pesticide usage in India was commenced in 1948 with DDT and Benzene Hexachloride (BHC) for malaria and locust control, respectively. India is currently the largest manufacturer of pesticides and the second-largest producer of agrochemicals in Asia. The average yearly pesticide demand in India is close to 90,000 MT.

Among the pesticides produced, endosulfan took the front seat by production as well as usage (Li and Macdonald 2005; Tiwari and Guha 2013). Endosulfan, a sulphurous acid ester of a chlorinated cyclic diol (Keith and Telliard 1979; Sunderam et al. 1992; Kimber et al. 1994), is an insecticide, classified under the category of Persistent Organic Pollutant (POP). It belongs to the organochlorine group. The compounds of this group are characterized by their high persistence in the environment, and their ability to bioaccumulate in animal tissues (Worthing 1983). Organochlorines, also known as chlorinated hydrocarbons, are insoluble in

M. Kumar · L. Philip (✉)
Environmental and Water Resources Engineering Division,
Department of Civil Engineering, Indian Institute
of Technology Madras, Chennai 600036, India
e-mail: ligy@iitm.ac.in

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_3

water and highly soluble in fat. Endosulfan, a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene sub-group, is a mixture of two stereo isomers alpha and beta endosulfan in a ratio of 7:3 (Martens 1976; Miles and Moy 1979). It is registered with several trademarks like cyclodan, thimol and malix. Pure endosulfan is a colorless crystal, but technical grade endosulfan is yellow to brown in color (Mukherjee and Gopal 1994; Penuela and Barcelo 1998). The molecular structure of alpha endosulfan and beta endosulfan has been shown Fig. 1. It is being used extensively throughout the world for the control of numerous insects in a variety of food/non-food crops and as a wood preservative. The physical and chemical properties of endosulfan are presented in Table 1.

Endosulfan is extremely toxic to fish and aquatic invertebrates and enters the air, water, and soil environments during its use and manufacture. The half-life of endosulfan in water and most fruits and vegetables is reported to be three to seven days (Mansingh and Wilson 1995; Miles and Pfeuffer 1997). Despite its rapid degradation in water, endosulfan can persist for a relatively long period when bound to soil particles, which can be a source of water contamination (Kennedy et al. 1994). Half-life of endosulfan in sandy loam is reported to be between 60 and 800 days (Kathpal et al. 1997). Though desorption potential of soil adsorbed endosulfan is less, there is a great chance of immediate run-off after application. POPs are highly stable and chemically non-reactive under common conditions. POPs persist in the environment, accumulate in the fatty tissues of living organisms and are harmful to human and wildlife. POPs have long half-lives in soils, sediments, air or biota and are typically "water-hating" and "fat-loving" chemicals, i.e. hydrophobic and lipophilic. In aquatic systems and soil, they partition strongly to

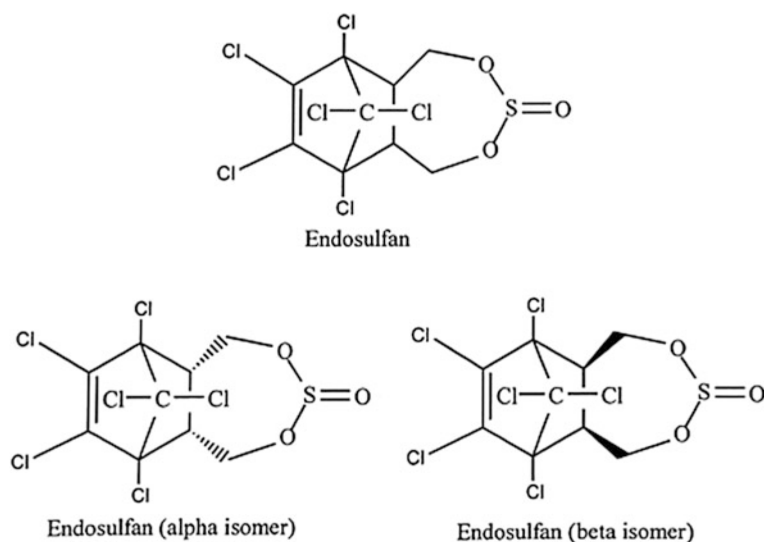


Fig. 1 Molecular structures of endosulfan and its two stereo Isomers

Table 1 Physical and chemical properties of endosulfan

Character	Description
Common name	Endosulfan
Trade names	Afidan, Beosit, Cyclodan, Devisulfan, Endocel, Endocide, Endosol, FMC 5462, Hexasulfan, Hildan, Hoe 2671, Insectophene, Malix, Phaser, Thiodan, Thimul, Thifor, and Thionex
Chemical class	Chlorinated hydrocarbons
Type	Organochlorine insecticide, nomaticide and acaricide
Chemical name	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Formulation	Formulations of endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets
Form	Colorless powder and Yellow to Brown crystalline solid
Composition	Mixture of alpha and beta isomers of endosulfan in the ratio of 70:30
Molecular formula	$C_9H_6Cl_6O_3S$
Molecular weight	406.9
Solubility	Water: 0.32 mg L^{-1} at 22 °C Ethanol: 65 g L^{-1} Hexane: 24 g L^{-1}
Melting point	70–100 °C
Vapour pressure	1200 Mpa at 80 °C
Stability	Persist longer under acidic conditions. Hydrolyzed slowly by water, more rapidly by acids and bases
Mode of action	Central nervous system stimulant. Acts as a contact and stomach poison in a wide variety of insects and mites
Use	Used as a pre-harvest insecticide and can be used as a wood preservative

solids, notably organic matter, avoiding the aqueous phase. Through the volatilization and condensation cycle, seepage and run-offs, pesticides get into surface water, groundwater, sea, ocean and the arctic environment (Tanaba et al. 1994; Carter 2000; Weber et al. 2010). The analysis of organochlorine pesticides in water, sediments, marine species and their products, such as cod liver oil, has been used to assess the levels and patterns of pollution of the marine environment (Krahn et al. 1997; Monirith et al. 1999; Zhulidov et al. 2000). Also, the nature of bioaccumulation makes the gateway for the food contamination. Endosulfan and many other pesticides residues were traced in fishes, mammals, water bodies, soil and even in human milk. Indiscriminate discharge of pesticide manufacturing/formulating industrial effluents on to the surrounding land has resulted in tremendous soil pollution leading to a drastic fall in productivity.

Owing to their effects on target as well as non-target organisms, lot of restriction was imposed on pesticide use. Many national and international environmental organisations have put up very stringent limits for pesticide presence in natural waters. As per Bureau of Indian Standards (BIS), the pesticide residues must be absent in drinking water. The existing treatment methods, employed by the pesticide manufacturing/formulating industries, failed to meet the effluent standards. At the same time, the development of a method for the complete detoxification of endosulfan in water and soil is still awaited.

The increasing concern about soil quality has generated a genuine interest in developing techniques to remove POPs from soil. In case, this can be achieved, total removal has to be our choice. Remediation methods, such as venting, solvent flushing, sparging, leaching, washing, thermal desorption and many more, are available to remove the pesticides from soil (Verstraete and Devliegher 1996). However, these methods transfer the pollutant from one system to another i.e. soil phase to liquid phase. Thus, bioremediation is an environmental-friendly and suitable alternative for the conventional treatment options. Various recalcitrant pollutants like highly energetic chemicals 2,4,6-Trinitrotoluene—TNT, hexahydro-1,3,5-trinitro-1,3,5-triazine—RDX and octahydro—1,3,5,7—tetranitro - 1,3,5,7—tetrazocine—HMX (Hawari et al. 2000), pentachlorophenol (Karamanev and Samson 1998), and polyaromatic hydrocarbons—PAHs (Ressler et al. 1999) have been reported to disappear after bioremediation. Despite the biotransformation of the POPs in almost all reported studies, little or no mineralization occurred and the fate of the compounds and their (bio) transformed products has not been evaluated. Consequently, several authors have addressed the importance of understanding the mechanisms of interactions of the POPs and their degradation products with matrix i.e. soil, organic matter and biomass (Achtnich et al. 1999; Knicker et al. 1999).

Endosulfan contamination is observed in the environment at considerable distances from the point of its original applications, because of its widespread usage and rapid transport. The presence of endosulfan residues in Indian estuaries i.e. Cochin estuary and Hugli estuary was reported by Sujatha et al. (1999) and Bhattacharya et al. (2003), respectively. In addition, endosulfan residues were detected in the atmosphere, soils, sediments, surface and subsurface waters, and food stuffs (Berrakat et al. 2002; Cerejeira et al. 2003; Golfinopoulos et al. 2003). As endosulfan is also found in groundwater, it is evident that there could be a high mobility of this chemical through the soil. All the physico-chemical methods reported for the remediation of endosulfan contaminated soil are either energy intensive and/or the sludge produced by the chemical processes requires further attention, i.e. they must be disposed into chemically secured landfills or subjected to further extensive treatments. Thus, these methods are neither economically viable nor environmentally friendly. The ultimate solution for the abatement of such chemicals which were considered to be recalcitrant, is the application of environmental biotechnology i.e., bioremediation using selected microbes (Siddique et al. 2003).

2 Microbial Degradation of Endosulfan

Bioremediation is employed for the successful decontamination of many pollutants, such as pentachlorophenol, diesel oil, herbicides, and polyaromatic hydrocarbons. A few studies were reported on the enrichment/isolation of bacterial and fungal cultures for the biodegradation of endosulfan. However, the mechanism of endosulfan biodegradation in water and soil, the fate of the (bio) transformed products and their consequential toxicity were not well understood in the past. Moreover, no information is available on the effect of environmental conditions on microbial population change and biodegradation pathway of endosulfan. So far, no data are available on the amount or the effects of degradation products resulting from the breakdown of endosulfan. Also, the previous studies did not emphasize the behavior of pure cultures of a bacterial consortium on degradation of endosulfan and its metabolites. The term biodegradation is used to describe a variety of different microbial processes that occur in the natural systems, such as mineralization, detoxification, co-metabolism, or activation (Alexander 1980). The process of bioremediation enhances the rate of natural microbial degradation of contaminants by microbes supplemented with nutrients, carbon sources or electron donors (Catallo and Portier 1992). This is possible by using indigenous microorganisms or by adding an enriched culture of microorganisms that have specific characteristics to degrade the desired contaminant at a quicker rate (Madsen 1991). In a bioremediation process, heterotrophic microorganisms break down hazardous compound as a substrate to obtain chemical energy. Hence organic pollutants can serve as carbon source, energy and nutrient sources for the microbial growth (Siddique et al. 2003). Ideally, bioremediation results in complete mineralization of contaminants to H_2O, CO_2 and other environmentally friendly byproducts without building up of intermediates (Lee et al. 1988).

Bioremediation processes generally rely upon the stimulation and natural selection of indigenous microorganisms in the soil or groundwater (Sims and Bass 1984). However, the natural soil flora may not have the metabolic capability to degrade certain compounds or classes of compounds or to emulsify the water-insoluble compounds (Riser-Roberts 2002). In such cases, bioaugumentation i.e. the supplementation of microorganisms plays a major role in the detoxification of these compounds (Baud-Grasset and Vogel 1995). Bioremediation processes can be broadly categorized into two groups: ex situ and in situ. Ex situ bioremediation technologies include bioreactors, biofilters, land farming and some composting methods (Riser-Roberts 2002), while in situ bioremediation technologies include bioventing, biosparging, biostimulation, liquid delivery systems and some composting methods. In situ treatments tend to be more attractive to vendors and responsible parties, because they are economical and eco-friendly to the environment (Lee and Ward 1985). However, the difficulties associated with in situ processes have limited their application in the field.

2.1 Microorganisms Used in Endosulfan Degradation

Microorganisms have been increasingly investigated as a source for degrading enzymes of xenobiotics (Chen and Mulchandani 1998). Several studies have reported the isolation of bacterial co-cultures (Awasthi et al. 1997) and mixed bacterial cultures (Sutherland et al. 2000; Kumar and Philip 2006a; Kumar et al. 2007) capable of degrading endosulfan. Mukherjee and Gopal (1994) reported the degradation of β-endosulfan by *Aspergillus Niger*. In addition, *Trichoderma harzianum* (Katayama and Matsumura 1993), *Phanerochate chrysosporium* (Kullman and Matsumura 1996) and *Mucor thermohyalospora* MTCC 1384 (Shetty et al. 2000) were used for endosulfan degradation; although these fungi were isolated for the degradation other compounds. An overview of endosulfan biodegradation experiments conducted with bacterial and fungal cultures are listed in Table 2.

Detoxification of endosulfan through biological means is receiving serious attention as an alternative to existing methods, such as incineration and landfill (Sutherland et al. 2000; Siddique et al. 2003). Some studies have described endosulfan as a sulfur source for microbial growth and a poor biological energy source when used as a sole carbon source (Guerin 1999; Sutherland et al. 2000). However, many studies emphasized that endosulfan can be biodegraded effectively when utilized as a carbon source (Awasthi et al. 1997; Siddique et al. 2003). In general, endosulfan can be degraded by attacking the sulfide group by oxidation and/or hydrolysis reactions.

2.2 Endosulfan Biodegradation by Hydrolysis

Hydrolysis is a bond breaking and bond forming process in which a molecule R–X, where X is a leaving group and reacts with water (H_2O) or hydroxide ion (OH^-) forming a new R–O bond and cleaving a R–X bond in the original molecule. The products of hydrolysis reaction are usually less of an environmental concern than the parent compounds, because they are usually transformed into more polar compounds, which are less hydrophobic than the original molecules (Martens 1976). Hydrolysis is a suitable pathway for the biodegradation of endosulfan in water and is greatly governed by pH and temperature (Sutherland et al. 2000). Both isomers of endosulfan are susceptible to alkaline hydrolysis (Goebel et al. 1982). Beta endosulfan hydrolyzes faster than the alpha endosulfan. This has been attributed to less steric hindrance from the S=O bond of the beta-isomer structure, leading to more susceptibility for nucleophilic (OH^-) to attack at the sulphur atom (Goebel et al. 1982).

Table 2 Microbial degradation of α-endosulfan (α-ES), β-endosulfan (β-ES) and its toxic metabolite, endosulfans sulfate (ESS), Endo ether (EE) and Endo lactone (EL)

Microorganism	Compound	Initial concentration (mg L^{-1})	Maximum degradation rate (mg L^{-1} d)	Cell density	References
Pseudomonas aeroginosa	ES	50	4.00	2.7–2.8[b]	Narkhede et al. (2015)
Agrobacterium tumefaciens	α-ES	70	15.83	0.7–0.8[c]	Thangadurai and Suresh (2014)
	β-ES	30	7.67	0.7–0.8[c]	
Alcaligenes faecalis JBW4	α-ES	70	12.24	1.9[c]	Kong et al. (2013)
	β-ES	30	5.04	1.9[c]	
Aspergillus sydoni	α-ES	70	3.69	0.07[g]	Goswami et al. (2009)
	β-ES	30	1.62	0.07[g]	
Acromobacter xylosoxidans	α-ES	70	3.95	0.7[c]	Li et al. (2009)
	β-ES	30	1.73	0.7[c]	
Mixed culture (*Stenotrophomonas maltophilia* and *Rhodococcus erythropolis*)	α-ES	70	3.41	1.2[d]	Kumar et al. (2007)
	β-ES	30	1.62	1.2[d]	
Mixed bacterial culture (*Bacilus circulans*-I; *Bacillus circulans*-II and *Staphylococcus*)	α-ES	70	1.07	ND[c]	Kumar and Philip (2006a)
	β-ES	30	0.458	ND[c]	
Mixed bacterial culture (*Bacilus circulans*-I; *Bacillus circulans*-II and *Staphylococcus*)	ESS	5	Nil	ND[c]	Kumar and Philip (2006b)
	EE	5	0.35	ND[c]	
	EL	5	0.352	ND[c]	
Klebsiella oxytoca KE-8	α-ES	121	11.14	ND[c]	Kwon et al. (2005)
	β-ES	29	1.71	ND[c]	
	ESS	173	9.36	ND[c]	
Klebsiella pneumoniae KE-1	α-ES	65	6.17	0.9[b]	Kwon et al. (2002)
	β-ES	28	2.55	0.9[b]	
Fusarium ventricosum	α-ES	70	14.2	ND[c]	Siddique et al. (2003)
	β-ES	30	6.6	ND[c]	
Pandoraea sp.	α-ES	70	8.19	0.52[d]	
	β-ES	30	3.18	0.52[d]	
Chlorococcum sp.	α-ES	4.24	0.13	ND[c]	Sethunathan et al. (2004)
	ESS	8.44	0.12	ND[c]	
Scenedesmus sp.	α-ES	4.24	0.14	ND[c]	
	ESS	8.44	0.05	ND[c]	
Anabaena flosaquae	α-ES	10	2.25 (4.0)[a]	50[e]	Lee et al. (2003)
Anabaena sp. PCC7120	α-ES	10	2.25	50[e]	
Bacillus sp. co-culture	α-ES	388	29.7	2×10^6(cfu)[f]	Awasthi et al. (2003)
	β-ES	386	27.7	2×10^6(cfu)[f]	

(continued)

Table 2 (continued)

Microorganism	Compound	Initial concentration (mg L^{-1})	Maximum degradation rate (mg L^{-1} d)	Cell density	References
Mycobacterium sp. ESD	β-ES	125	<30	0.78[g]	Sutherland et al. (2002b)
Enriched culture	ESS	125	25	0.4[g]	Sutherland et al. (2002c)
	α-ES	125	17.8	0.4[g]	
	β-ES	125	12.5	0.4[g]	
Phanerochaete chrysosporium	α-ES	1	0.48	ND[c]	Kullman and Matsumura (1996)

[a]pH controlled culture (pH 7.2)
[b]$OD_{550\ nm}$
[c]Not determined
[d]$OD_{600\ nm}$
[e]Chlorophyll concentration (mg L^{-1})
[f]Initial cell concentration
[g]$OD_{595\ nm}$

2.3 Endosulfan Biodegradation by Oxidation

In oxidation process, oxygen molecules readily combine with the S=O bond of the endosulfan and oxidized to SO_2 to form endosulfan sulfate ($C_9H_6Cl_6O_4S$), which is more of environmental concern than the parent compound. This may be due to the high non-polarity (water solubility of 0.22 mg l^{-1}) and increased molecular weight (422.9) of endosulfan sulfate (Martens 1976; Kullman and Matsumura 1996). Therefore, endosulfan sulfate persists in the environment for a longer period than the parent compound (Kaur et al. 1998; Ghadiri and Rose 2001).

2.4 Aerobic and Anaerobic Degradation of Endosulfan

The environmental fate of endosulfan in water and soil has been studied by several workers in order to design a bioremediation process. The difference in the persistence of the two isomers (alpha and beta isomers of endosulfan) has been attributed to various factors, such as their differential volatilization, phyto decomposition, alkaline hydrolysis, as well as their biotic metabolism (Cotham and Bidleman 1989). Endosulfan undergoes biotransformation in soil to form endosulfan sulfate - one of the major metabolites of endosulfan under aerobic conditions (Stewart and Cairns 1974; Martens 1976; Kathpal et al. 1997). Endosulfan sulfate is nearly more toxic compared than parent chemical and it does not undergo any further degradation. Therefore, its residues tend to persist and increase in the environment

(Coleman and Dolinger 1982; Kullman and Matsumura 1996; Sutherland et al. 2000; Kim et al. 2001). But, in contradiction a few researchers have worked on endosulfan sulfate degradation using enriched bacterial cultures (Sutherland et al. 2002a; Kwon et al. 2005). As of now there are reports of direct hydrolysis of endosulfan sulfate to endodiol by soil inocula (Miles and Moy 1979).

Under low oxygen or anaerobic conditions, such as sea water, which sediments or sludge, endosulfan is rapidly metabolized by sulfur-separation reaction which results in the release of the sulfur moiety. Removal of the sulfur moiety of endosulfan drastically decreases the toxicity of endosulfan and the metabolites are readily degraded by a variety of microbes (Goebel et al. 1982). In anaerobic condition, endosulfan can be degraded by hydrolysis pathway, leading to the formation of endosulfan diol, which is usually less toxic than the endosulfan isomers (Guerin 1999). Endosulfan diol can be further degraded to endosulfan lactone, endosulfan hydroxyether and endosulfan monoaldehyde and dialdehyde by a wide range of bacterial and fungal cultures in the anaerobic condition (Martens 1976; Kullman and Matsumura 1996; Awasthi et al. 1997, 2003; Sutherland et al. 2000). Sutherland et al. (2000) reported that the terminal metabolite i.e. endosulfan monoaldehyde, was further metabolized by a mixed anaerobic culture into polar products. The absence of endosulfan in any system doesn't really indicate the complete removal of the compound. Sometimes, the molecule was transformed to products that are not amenable to detection under a given set of analytical conditions, but gives no clue of the extent to which the compound was transformed. In contrast, an increase in chloride ions concentration, in the anaerobic system clearly, indicates the extensive degradation of endosulfan molecule. The general stoichiometry of endosulfan degradation can be proposed as follows:

$$C_9H_6Cl_6O_3S + 6H_2O \rightarrow 3CO_2 + 6CH_2 + 6Cl^- + 6H^+ + SO_3^- \quad (1)$$

The stoichiometric chloride concentration from Eq. (1) can be compared with the measured chloride ion concentration in the system. The chloride mass balance and the extent of endosulfan absence in the system can really reflect the endosulfan degradation/mineralization.

3 Mechanism, Pathways and Involvement of Enzymes in Endosulfan Degradation

A wide variety of microorganisms, mostly bacteria, a few fungi and cynobacteria were utilized for endosulfan biodegradation (Kullman and Matsumura 1996; Awasthi et al. 1997; Sutherland et al. 2002b; Kumar and Philip 2006a; Kumar et al. 2007; Goswami et al. 2009; Kataoka et al. 2011; Thangadurai and Suresh 2014; Kumari et al. 2014; Abraham and Silambarasan 2014; Narkhede et al. 2015) under aerobic and/or anaerobic conditions. As discussed earlier, the pathway of

endosulfan degradation in aerobic condition is entirely different from that of anaerobic condition. On the other hand, many investigations reported the usage of endosulfan as carbon/energy source and/or sulfur source.

Kullman and Matsumura (1996) assessed the enzymatic mechanisms of endosulfan metabolism by white rot fungi *Phanerochaete chrysosporium* under ligninolytic (nutrient deficient) and non-ligninolytic (nutrient-rich) culture conditions. Rapid metabolism of endosulfan occurred under each nutrient condition tested. However, the extent of degradation and the nature of the metabolic products differed for nutrient-deficient and nutrient-rich media. The major endosulfan metabolites, as identified in the system, were endosulfan sulfate, endosulfan diol, endosulfan hydroxyether, and an unknown metabolite (tentatively identified as endosulfan dialdehyde). From the nature of the metabolites formed, it can be inferred that endosulfan was degraded by the cultures following both oxidative and hydrolytic pathways. The pathway of endosulfan degradation by *Phanerochaete chrysosporium* is shown in Fig. 2.

Sutherland et al. (2002b) investigated the feasibility of oxidative detoxification of beta endosulfan using gram-positive bacterium *Mycobacterium* sp. strain ESD. The strain was able to use endosulfan as a sole source of sulfur for growth. *Mycobacterium* sp. strain ESD metabolized the endosulfan isomers to endosulfan sulfate and endosulfan monoaldehyde. However, the alpha isomer was predominantly converted to endosulfan sulfate, and the beta isomer to endosulfan monoaldehyde. From the results, they observed that the formation of both endosulfan sulfate and endosulfan monoaldehyde by *Mycobacterium* sp. strain ESD

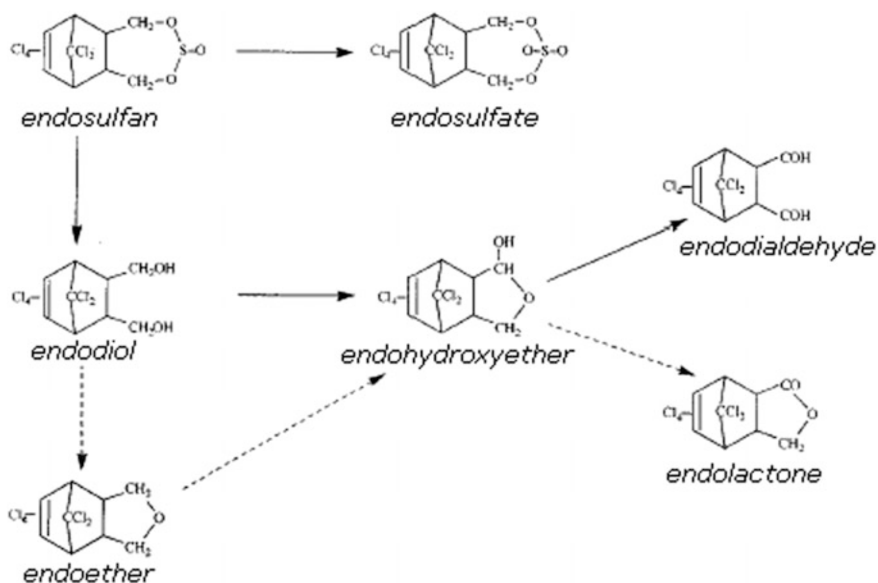


Fig. 2 Pathway of endosulfan degradation by *Phanerochaete chrysosporium*

occurred in response to sulfur starvation. They also reported that the formation of above metabolites did not occur at relatively lower levels of inorganic sulfate or sulfite. Later, mono-oxygenase enzymes isolated from *Mycobacterium* sp. strain ESD was employed for beta endosulfan biodegradation experiments. It was observed that beta isomer was converted into endosulfan monoaldehyde and endosulfan hydroxyether, but alpha endosulfan and endosulfan sulfate were not degraded.

Earlier investigations reveal that the major metabolites, formed under the aerobic biodegradation of endosulfan, were endosulfan sulfate, endosulfan diol, endosufal ether, endosulfan ether, endosulfan hydroxy ether, endosulfan lactone and endosulfan monoaldehyde. Different degradation rates for both parent as well as degradation intermediates were observed, however, the production of metabolites was found to be species-specific. Moreover, it is well accepted that consortium of microbial strains were more effective in the degradation of endosulfan than the pure cultures. The consolidated key pathways, involved in the biotransformation/biodegradation of endosulfan, are given in Fig. 3 (Thangadurai and Suresh 2014).

The production of endosulfan diol is an important step in the detoxification of endosulfan via hydrolysis. On the other hand, the formation of endosulfan hydroxyether is mainly due to the result of oxidation of its methylene groups.

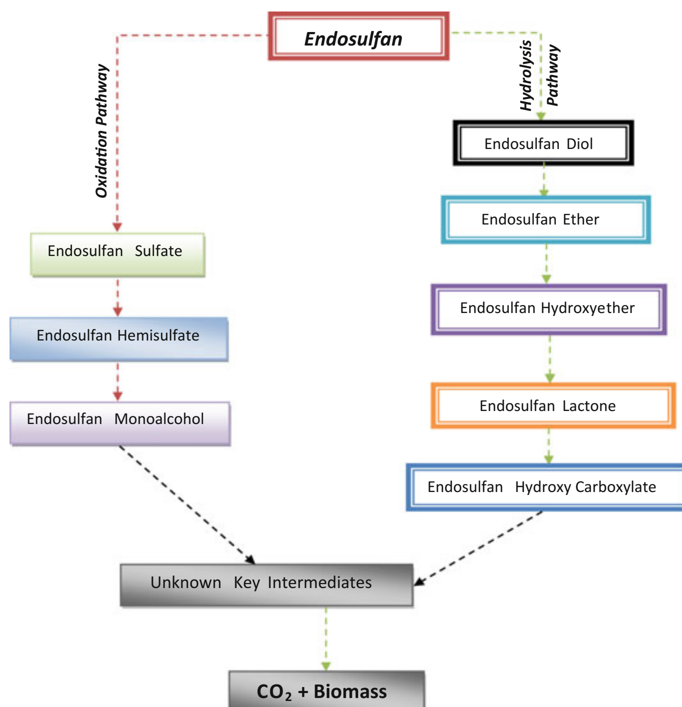


Fig. 3 Endosulfan biotransformation pathways

Several studies have reported that endosulfan sulfate was difficult to be degraded as compared to the parent compound, where as other studies reported the disappearance of endosulfan sulfate with time due to the bacterial activity. In the past, several aqueous phase, soil-slurry phase and soil phase endosulfan biodegradation/ biomineralization investigations were carried out. However, the aqueous and soil phase microbial degradations of endosulfan are completely different owing to the properties of pollutants and the characteristics of soil, such as clay content, organic matter content, soil texture and available moisture. Tiwari and Guha (2013) reported that the degradation rate of both the isomers of endosulfan was slowed down in soil phase compared to aqueous phase. The decrease in degradation rate of endosulfan isomers in soil was mainly due to the partition coefficient of the compound.

4 Factors Affecting Degradation Process of Endosulfan

Many researchers emphasized on the impact of pH, moisture content, presence of additional/supplementary carbon source, size of inoculum and initial pesticide concentration in the soil during biodegradation of pesticides (Sims and Bass 1984; Goldstein et al. 1985). It was also reported that development of effective and suitable bioremediation technology relied on the efficacy of the soil bacteria or the augmented bacterial culture (Awasthi et al. 1997). The influence of the above mentioned parameters during biodegradation of various other pesticides were also reported by many researchers (Block et al. 1993; Kaur et al. 1998), however, a few studies were only carried out to assess the influence of these factors on endosulfan biodegradation.

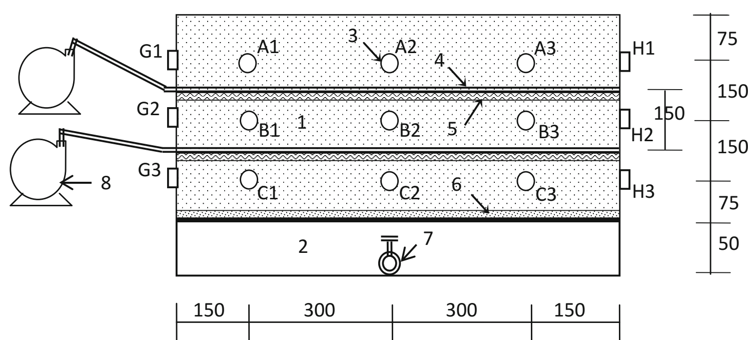
4.1 Effect of Moisture Content

The persistence or dissipation and degradation of pesticides in soil mainly depend on the moisture content of the soil regime (Ghadiri and Rose 2001). All the successful endosulfan biodegradation studies, conducted so far, were carried out at a moisture content greater than 30 % (Kumar and Philip 2006b; Kumar et al. 2008; Kataoka et al. 2011; Ali et al. 2014). It indicates that a minimum moisture content of 30 % is essential in soil biodegradation experiments to make the endosulfan bioavailable through film diffusion. Moreover, it is reported that endosulfan degradation was faster in flooded soils than in non-flooded soils (Singh et al. 1999; Awasthi et al. 2000). In contrast, degradation of alpha endosulfan was more readily observed under non-flooded condition (degradation of around 45 %) than the flooded condition (degradation of around 15–18 %) during 120 days of incubation (Sethunathan et al. 2002). Tiwari and Guha (2013) reported that the rate of degradation of endosulfan isomers slowed down in soil phase compared to aqueous

phase due to the higher k_w/k_s values. The effect was more pronounced in the case of beta-isomer than to alpha-isomer, due to an increase in partition coefficient of beta-isomer compared to the later (1.8 times higher).

In agricultural fields, endosulfan is mainly bound to soil residues at a depth of around 300–500 mm (Ismail et al. 2002). For any in situ operation, area of soil to be treated may not cause any operational difficulty as far as bioremediation is concerned, whereas the depth is a deciding factor in the success of in situ bioremediation system along with other operational parameters, such as moisture content, pH, inoculum size and so on. Kumar and Philip (2007) conducted a soil phase endosulfan (0.78 mg g^{-1} of soil) biodegradation study in a pilot scale bioreactor having a soil volume of 275 kg (Fig. 4). During the study, the loss in moisture content was observed as a result of bacterial action and due to environmental conditions. The variation in moisture content in the pilot scale reactor is shown in Fig. 5.

However, the loss in moisture content was compensated by with the addition of nutrient medium to increase or sustain the bacterial activity. However, it is also emphasized that the supply of more quantity of moisture can result in the transport of endosulfan rather than biodegradation.

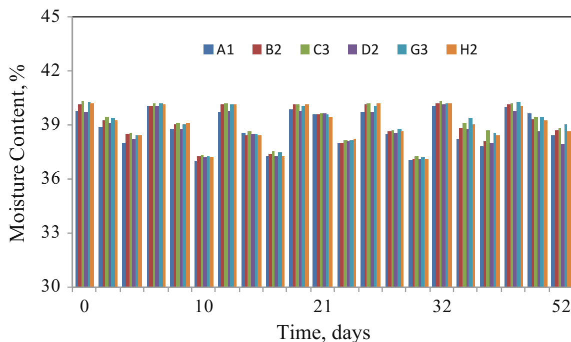


All dimensions are in mm

1. Soil reactor portion 2. Leachate chamber 3. Sampling ports (A1, B1, C1 etc. are soil sample collection ports and on the rear side numbered as D1, E1 and F1 respectively for rows 1, 2 and 3) 4. Nutrient supply pipes 5. Sand layer 6. Supporting medium 7. Leachate collection tap 8. Peristaltic pump.

Fig. 4 Schematic diagram of the pilot scale reactor (Kumar and Philip 2007)

Fig. 5 Moisture variation in the pilot scale soil bioreactor reactor during operation



4.2 Effect of Supplementary Carbon

In the past, several attempts were made to understand the role of endosulfan as a growth promoter or growth suppresser while using carbon, energy and sulfur source (Awasthi et al. 1997; Kumar and Philip 2006a; Goswami et al. 2009; Li et al. 2009; Kataoka et al. 2011; Thangadurai and Suresh 2014). The factors, like the presence of co-pollutant and/or supply of external carbon source to the enriched microbial consortia, have some indirect effect on biodegradation process (Guerin 1999; Awasthi et al. 2000). Many researchers observed that the addition of auxiliary carbon to the system having xenobiotic compounds increased the biodegradation potential of bacterial and fungal cultures (Topp et al. 1996; Kumar and Philip 2006a). A steady-increase in bacterial growth as well as endosulfan biodegradation was reported in the presence of dextrose at low levels, i.e. 1 g L^{-1} (Fig. 6), whereas no improvement in the degradation of alpha and beta endosulfan was observed when dextrose concentration was increased beyond 1 g L^{-1} (Kumar and Philip 2006a).

In contradiction, Awasthi et al. (2000) reported that the addition of glucose has not increased the degradation efficiency of endosulfan. Similarly, a decrease of 17 % alpha-isomer and 16 % of β-isomer endosulfan degradation efficiency was observed after 18 d of incubation in the presence of sucrose (Goswami et al. 2009). In many other studies also, the presence of more favorable carbon sources have been shown to impede the degradation of less favorable chemicals (Holtel et al. 1994). This could be due to the mechanism of catabolite repression or decrease in the rates of transcription either due to super coiling of promoter DNA or by decreased binding of transcription factors (Botsfold and Harman 1992; Holtel et al. 1994). However, this fact may be related to the metabolism of the specific culture that degrades the target pollutant.

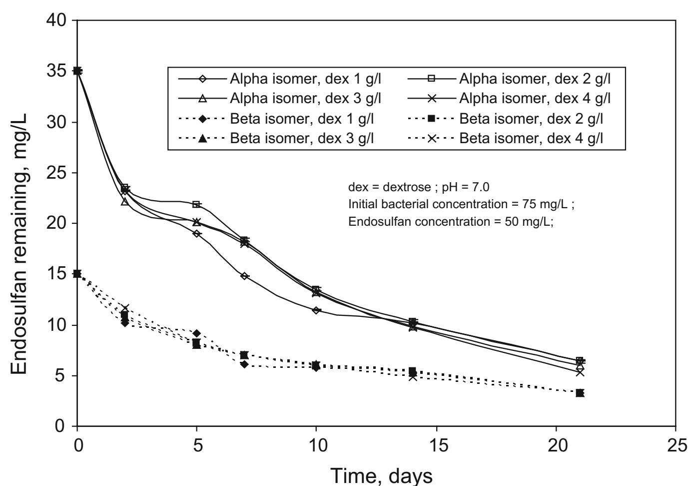


Fig. 6 Endosulfan degradation at various dextrose concentrations in aerobic system

Kataoka et al. (2011) conducted a soil-applied endosulfan biodegradation study in the presence of wheat bran and cane molasses using *Mortieralla* sp. Both these substrates are used as an energy source for the growth of fungi or yeast. It was observed that the degradation of α- and β-isomers of endosulfan was different with wheat bran and cane molasses, separately, despite sufficient mycelial growth on both substrates. However, a very low level of degradation was achieved using cane molasses as a substrate.

4.3 Effect of PH

The environmental fate and microbial degradation of endosulfan in water and soil is mainly influenced by the pH of the medium. The optimal pH for endosulfan degradation was observed as 8.5 (Awasthi et al. 2000). This could be due to the better bioavailability of endosulfan and optimal biotic activity of bacterial cells at this pH value. Also, it was observed that at alkaline pH, endosulfan was converted into endosulfan diol by alkaline hydrolysis (Guerin 1999) and it is difficult to differentiate from microbial degradation where the culturing leads to the increase in pH of the medium (Martens 1976; Miles and Moy 1979). In contradictory, some researchers observed that strains degrading high concentration of endosulfan have drastically increased the acidity of culture media to a pH level of 3.3 (Siddique et al. 2003).

On the other hand, no considerable degradation of endosulfan was observed at pH 5 which favored the formation of endosulfan sulfate (Awasthi et al. 2000). Kumar and Philip (2006a) carried out endosulfan biodegradation experiments using mixed bacterial culture at various pH values i.e. 4, 6, 7, 8 and 10 in both aerobic and facultative anaerobic conditions. At the end of 21 days, endosulfan degradation efficiency (at pH 4) was 55.7 ± 0.2 % under aerobic condition (Fig. 7). The increase in pH value to 6 and 7 increased the degradation efficiency to 71.3 ± 0.2 % and 71.58 ± 0.2 %, respectively. However, further, increase in pH value to 8 reduced the efficiency marginally (71.28 ± 0.2 %) and at pH 10, it was reduced drastically (37.56 ± 0.2 %). A decrease in pH to 4 and an increase in pH to 10 reduced the endosulfan degradation efficiency by 22.18 ± 1.4 % and 47.53 ± 1.45 %, as compared to neutral pH, after 21 days of incubation.

On the other hand, Narkhede et al. (2015) observed a drastic decrease in pH of the system from 7.2 to 4.98 in 288 h during aerobic endosulfan biodegradation study using *Pseudomonas aeroginosa*. A decrease in pH was due to the formation of by-products of endosulfan and/or due to the HCl and organic acids released by the microorganisms (Li et al. 2009; Castillo et al. 2011). However, they observed that highest endosulfan degradation was observed at pH 7.0. Siddique et al. (2003) also observed similar trends, while studying the endosulfan degradation by enriched fungal and bacterial strains under aerobic condition. This may be linked to the decreased growth of microbes at extreme pH. Interestingly, a significant increase in endosulfan degradation efficiency was observed at alkaline pH values, i.e. pH 8 and

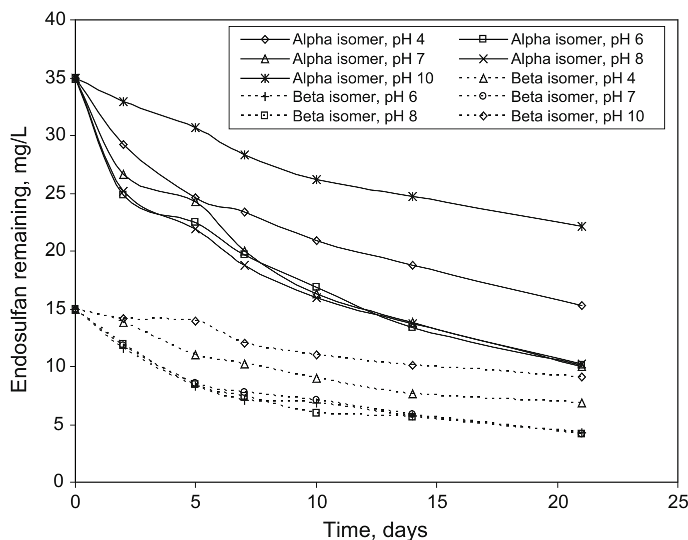


Fig. 7 Endosulfan degradation in aerobic system at different pH values

10 (Kumar and Philip 2006a). It could be due to the higher hydrolysis of endosulfan at the higher pH ranges (Guerin 1999; Kwon et al. 2002). However, the bacterial enzymes, segregated during the process of endosulfan biodegradation, play a vital role in the degradation of endosulfan and formation of its metabolites.

5 Microbial Toxicity of Endosulfan and Degradation Products

In the recent years, plant-associated nitrogen fixing bacteria have been considered as potential alternatives for inorganic nitrogen fertilizers for promoting plant growth and related yield. Ahmad et al. (2006) indicated the use of *A. Chroococuum* as a nitrogen fixing bacterium. The presence of toxic chemicals, i.e. pesticides including endosulfan, can affect the protection system of the bacterium against oxygen damage (mainly on bacterial membrane) and changes the membrane properties. This makes the protection system less effective. Moreover, the formation of protective cyst can be observed in the bacterium under stress conditions. Castillo et al. (2011) reported the effect of endosulfan on the membrane properties of the nitrogen fixing bacteria and cyst formation at an endosulfan concentration of 10 mg L^{-1}. Moreover, the addition of endosulfan substantially reduced the nitrogenase activity of *Azotobactter* sp. cultures in the aqueous phase degradation studies using a concentration of 10 mg L^{-1}. However, total inhibition was not observed under the test condition. The inhibition of nitrogenase enzyme by endosulfan on rhizosphere

of wheat was reported by Kalyani et al. (2010). Therefore, an increase in concentration beyond this levels or several folds higher dosage of endosulfan can completely damage the nitrogen fixing bacterial culture and their activity in the soil.

Dehydrogenase enzyme is an indicator enzyme for amendments, management practices and microbial activity in soil. These enzymes are mainly synthesized by the soil bacteria in response to pesticide pollution. An increase in the dehydrogenase levels in the soil can be taken as an indicator of the bioremedial action by the native species (Bhalerao 2012; Narkhede et al. 2015). Moreover, an increase in the activities of sulfatase and dehalogenase can be used to quantify the bioremedial action of the species. The higher concentration of endosulfan as well as endosulfan sulfate has the capability to damage such activities and inhibit the biodegradation process. So far, no study has reported the maximum level of endosulfan and endosulfan sulfate that can destroy the above enzyme activities.

On the other hand, growth response of cyanobacterial strains in presence of endosulfan was found to be inhibitory, but extent of inhibition was more damaging beyond 7.5 mg L^{-1} of endosulfan. The reduction in growth and damaging effect of endosulfan on cyanobacteria may be due to the decrease in photosynthesis ability of chlorophyll a. The site of action of endosulfan inhibition is closely associated with PSII, such as non-cyclic electron acceptor in the electron transport system (Kumar et al. 2008). The above observations indicate that endosulfan exerts its toxic effect through free radicals formed in the oxidative stress. In general, photosynthetic organisms counteract the toxicity of chemicals/pesticide induced free radicals by increasing their antioxidative defence mechanisms, which include enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and low molecular weight compounds such as ascorbate, glutathione, flavonoids, tocopherols, and carotenoids. Kumar et al. (2008) observed the bacterial resistance to endosulfan through antioxidant enzymes as well as through osmoprotectant, i.e. proline. On the other side, it has been reported that endosulfan could damage the mechanism of MAP Kinases signalling, mitochondrial dysfunction and lipid peroxidation. However, the metabolites of endosulfan, such as endo diol, endo ether, endo hydroxyether and endo lactone, have very little toxicity on the bacterial cultures (Shivaramaiah et al. 2005).

6 Future Perspectives

The investigations carried out in the past show that microbial cultures as cells and cell free extracts (i.e. their enzymes) have great potential in endosulfan biodegradation and bio-mineralization. In the past, most of the soil biodegradation studies were conducted in very small reactors with homogenous mixture of soil and endosulfan. But the condition of endosulfan-contaminated site is entirely different from the laboratory conditions. Therefore, more focus should be given on the actual prevailing condition at the contaminated site. In addition, other successful remediation methods, such as bio-venting, bio- sparging and combination of methods

(i.e. bio-electro chemical methods) can be tested for their feasibility of endosulfan biodegradation or bioremediation of endosulfan contaminated soil systems. On the other side, the formation of intermediate compounds of endosulfan is culture specific, but most of the researchers try to stop the formation of endosulfan sulfate by changing the culture growth conditions. In order to understand more detailed information about endosulfan biodegradation, it is essential to investigate the following points in the future.

- Most of the endosulfan degradation experiments (in aqueous systems) were conducted at high endosulfan concentrations, certainly to represent the endosulfan concentration which is normally discharged from pesticide formulating industries. But, the concentration of endosulfan residues observed in surface and groundwater is very less as compared to industrial effluents. Hence, degradation studies can be conducted at actual endosulfan concentrations which are commonly found in surface and groundwater systems.
- Most researchers pointed out that the endosulfan and its metabolite biodegradation is associated with several bacterial enzymes, which can be identified and characterized to promote the endosulfan degradation.
- Mostly, multiple pollutants are present in the contaminated aqueous (i.e. water, wastewater) and soil systems. Therefore, the effect of other pollutants has to be investigated before designing a bioremediation system for the field application.
- The biotoxicity of endosulfan metabolites to various other species, like earth worms and nitrogen fixing bacteria, need to be explored in detail.
- Studies correlating the geological/geophysical characteristics of the contaminated site to bacterial endosulfan degradation need to be carried out.
- A mathematical model incorporating fate, transport and biodegradation of endosulfan can be developed to understand the transport of endosulfan in the soil systems.

7 Conclusions

Traces of endosulfan residues were reported in many parts of the world. Biodegradation and bioremediation of recalcitrant pesticides including endosulfan is a challenging task that needs deep investigation of the site specific and target compound specific information. The selection and/or identification of suitable microbe(s) and their growth optimization play a major role in bioremediation process. In case of endosulfan biodegradation, facultative anaerobic condition was found to be the best suitable condition, which shows no formation of endosulfan sulfate. The mixed bacterial culture has a huge potential in biodegradation rather than pure bacterial strains. The toxicity of endosulfan and endosulfan metabolites is still poorly understood and requires further investigation. Therefore, combining biotoxicity analysis, enzymatic degradation of endosulfan and correlating the

site-specific and bacterial-specific data need to be generated to accelerate the degradation process of endosulfan in soils and aquatic systems.

Acknowledgments The authors thank IIT Madras for providing necessary facilities to complete this assignment on time.

References

Abraham, J., & Silambarasan, S. (2014). Biomineralization and formulation of endosulfan degrading bacterial and fungal consortiums. *Pesticide Biochem Physiology, 116*, 24–31.

Achtnich, C., Sieglen, U., Knackmuss, H. J., & Lenke, H. (1999). Irreversible binding of biologically reduced 2,4,6-trinitrotoluene to soil. *Environmental Toxicology and Chemistry, 18*, 2416–2423.

Ahmad, F., Ahmad, I., Aqil, F., Ahmed, W. A., & Sousche, Y. S. (2006). Plant growth promoting potential of free-living diazotrophs and other rhizobacteria isolated from Northern Indian soil. *Biotechnology Journal, 1*, 1112–1123.

Alexander, M. (1980). Biodegradation of chemicals of environmental concern. *Science, 211*, 132–138.

Ali, M., Kazmi, A. A., & Ahmed, N. (2014). Study on effects of temperature, moisture and pH in degradation and degradation kinetics of aldrin, endosulfan, lindane pesticide during full-scale continuous rotary drum composting. *Chemosphere, 102*, 68–75.

Awasthi, N., Ahuja, R., & Kumar, A. (2000). Factors influencing the degradation of soil applied endosulfan isomers. *Soil Biology & Biochemistry, 32*, 1697–1705.

Awasthi, N., Manickam, N., & Kumar, A. (1997). Biodegradation of endosulfan by a bacterial co-culture. *Bulletin of Environment Contamination and Toxicology, 59*, 928–934.

Awasthi, N., Singh, A. K., Jain, R. K., Khangarot, B. S., & Kumar, A. (2003). Degradation and detoxification of endosulfan isomers by a defined co-culture of two *Bacillus* strains. *Applied Microbiology and Biotechnology, 62*, 279–283.

Baud-Grasset, F., Vogel, T. M. (1995). Bioaugmentaion: Biotreatment of contaminated soil by adding adapted bacteria. In R. E. Hinchee, J. Fredrickson, B. C. Alleman (Eds.), *Bioaugmentation for site remediation*. *3rd international insitu on-site bioreclamation symposium*. Columbus, OH: Battelle Press.

Berrakat, A. O., Kim, M., Qian, Y., & Wade, T. L. (2002). Organochlorine pesticides and PCB residues in sediments of *Alexandria harbour*, Egypt. *Marine Pollution Bulletin, 44*, 1421–1434.

Bhalerao, T. S. (2012). Bioremediation of endosulfan-contaminated soil by using bioaugmentation treatment of fungal inoculant *Aspergillus Niger*. *Turkish Journal Boilogy, 36*, 561–567.

Bhattacharya, B., Sarkar, S., & Mukherjee, N. (2003). Organochlorine pesticide residues in sediments of a tropical mangrove estuary, India; Implications for monitoring. *Environment International, 29*, 587–592.

Block, R., Stroo, H., & Swett, G. H. (1993). Bioremediation: Why doesn't it work sometimes? *Chemical Engineering Progress, 89*, 44–50.

Botsfold, J. L., & Harman, J. G. (1992). Cyclic AMP in prokaryotes. *Microbiological Reviews, 56*, 100–122.

Carter, A. (2000). How pesticides get into water and proposed reduction measures. *Pestic Outlook*, 149–156.

Castillo, J. M., Casas, J., & Romero, E. (2011). Isolation of an endosulfan-degrading bacterium from a coffee farm soil: Persistence and inhibitory effect on its biological functions. *Science of the Total Environment, 412–413*, 20–27.

Catallo, W. J., & Portier, R. J. (1992). Use of indigeneous and adapted microbial assemblages in the removal of organic chemicals from soils and sediments. *Water Science and Technology, 25*, 229–237.

Cerejeira, M. J., Viana, P., Batista, S., Pereira, T., Silva, E., Valerio, M. J., et al. (2003). Pesticides in Portuguese surface and ground waters. *Water Research, 37*, 1055–1063.

Chen, W., & Mulchandani, A. (1998). The use of live biocatalysts for pesticide detoxification. *Trends in Microbiology, 16*, 71–76.

Coleman, P. F., & Dolinger, P. M. (1982). *Environmental health evaluation of California restricted pesticides*. (Endosulfan monograph no 4). Menlo Park, California, USA: PM Dolinger Associates.

Cotham, W. E., & Bidleman, T. F. (1989). Degradation of malathion, endosulfan and fevelarate in sea water/sediment in microcosms. *Journal of Agriculture and Food Chemistry, 37*, 824–828.

Ghadiri, G., & Rose, C. W. (2001). Degradation of endosulfan in a clay soil from cotton forms of western farms of Queensland. *Journal Environment Management*, *62*,155–169.

Goebel, H., Gorbach, S., Knauf, R. H., & Rimpau, Huttenbach. (1982). Properties, effects, residues and analysis of the insecticide endosulfan. *Residue Rev, 83*, 161–165.

Goldstein, R. M., Mallory, L. M., & Alexander, M. (1985). Reasons for possible failure of inoculation to enhance biodegradation. *Applied and Environment Microbiology, 50*, 977–983.

Golfinopoulos, S. K., Nikolaou, A. D., Kostopoulou, M. N., Xilourgidis, N. K., Vagi, M. C., & Lekkas, D. T. (2003). Organochlorine pesticides in the surface waters of Northern Greece. *Chemosphere, 50*, 507–516.

Goswami, S., Vig, K., & Singh, D. K. (2009). Biodegradation of α and β endosulfan by *Aspergillus Niger*. *Chemosphere, 75*, 883–888.

Guerin, T. F. (1999). The anaerobic degradation of endosulfan by indigenous microorganisms from low-oxygen soils and sediments. *Environmental Pollution, 106*, 13–21.

Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S., & Ampleman, G. (2000). Microbial degradation of explosives: biotransformation versus mineralization. *Applied Microbiology and Biotechnology, 54*, 605–618.

Holtel, A., Margues, S., Mohler, I., Jakubikz, U., & Timmis, K. N. (1994). Carbon source-dependent inhibition of xyl operon expression of the *Pseudomonas putida* TOL plasmid. *Journal of Bacteriology, 176*, 1773–1776.

Ismail, B. S., Enoma, A. O. S., Cheah, U. B., Lum, K. Y., & Malik, Z. (2002). Adsorption, desorption, and mobility of two insecticides in Malaysian agricultural soils. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 37*(4), 355–364.

Kalyani, S. S., Sharma, J., Dureja, P., Singh, S., & Lata. (2010). Influence of endsoulfan on microbial biomass and soil enzymatic activities of a tropical alfisol. *Bulletin of Environment Contamination Toxicology*, *84*, 351–356.

Karamanev, D., & Samson, R. (1998). High-rate biodegradation of pentachlorophenol by biofilm developed in immobilized soil bioreactor. *Environmental Science and Technology, 32*, 994–999.

Kataoka, R., Takagi, K., & Sakakibara, F. (2011). Biodegradation of endosulfan by *Mortieralla* sp. strain W8 in soil: Influence of different substrates on biodegradation. *Chemosphere, 85*, 548–552.

Katayama, A., & Matsumura, F. (1993). Degradation of organochlorine pesticides, particularly endosulfan by *Trichoderma harzianum*. *Environmental Toxicology and Chemistry, 12*, 1059–1065.

Kathpal, T. S., Singh, A., Dhankhar, J. S., & Singh, G. (1997). Fate of endosulfan in cotton soil under sub-tropical conditions of northern India. *Pesticide Science, 50*, 21–27.

Kaur, I., Mathur, R. P., Tandon, S. N., & Dureja, P. (1998). Persistence of endosulfan (technical) in water and soil. *Environmental Technology, 19*, 115–119.

Keith, L. H., & Telliard, W. A. (1979). Priority pollutants L: A perspective view. *Environmental Science and Technology, 13*, 416–423.

Kennedy, I. R., Ahmad, N., Truite, J., Kimber, S., Sebastian, S., & Lee, A. (1994). Transport and fate of pesticides in cotton production systems. In *Proceedings of the impact on the pesticides*

on the riverine environment using the cotton industry as a model. Second annual workshop, LWRRDC Occasional Paper 23/98, Canberra, Australia.

Kim, Y. K., Kim, S. H., & Choi, S. C. (2001). Kinetics of endosulfan degradation by Phanerochaete chrysosporium. *Biotechnology Letters, 23*, 163–166.

Kimber, S. W. L., Southan, S. K., Ahmad, N., & Kennady, I. R. (1994). The fate of endosulfan sprayed on cotton. In G. A. Constable, & N. W. Forrester (Eds.), *Challenging the future: Proceedings of the world cotton research conference-I. CSIRO* (pp. 589–594). Melbourne, Vic.

Knicker, H., Brunnus-Nagel, D., Drzyzga, O., Vonlow, E., & Steinbach, K. (1999). Characterization of ^{15}N-TNT residues after an anaerobic/aerobic treatment of soil/molasses mixtures by solid-state ^{15}N NMR spectroscopy. Determination and optimization of relevant NMR spectroscopic parameters. *Environmental Science and Technology, 33*, 343–349.

Kong, L., Zhu, S., Zhu, L., Xie, H., Su, K., Yan, T., et al. (2013). Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. *Journal Environment Science, 25*(11), 2257–2264.

Krahn, M. M., Becker, P. R., Tilbury, K. L., & Stein, J. E. (1997). Organochlorine contaminants in blubber of four seal species: integrating biomonitoring and specimen banking. *Chemosphere, 34*, 2109–2121.

Kullman, S. W., & Matsumura, F. (1996). Metabolic pathway utilized by *Phenerochete chrysosporium* for degradation of the cyclodine pesticide endosulfan. *Applied and Environment Microbiology, 62*, 593–600.

Kumar, S., Habib, K., & Fatma, T. (2008). Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. *Science of the Total Environment, 1–3*, 130–138.

Kumar, M., Lakshmi, V., & Khanna, S. (2007). Biodegradation and bioremediation of endosulfan contaminated soil. *Bioresource Technology, 99*, 3116–3122.

Kumar, M., & Philip, L. (2006a). Bioremediation of endosulfan contaminated soil and water—optimization of operating conditions in laboratory scale reactors. *Journal of Hazardous Materials, 136*(2), 354–364.

Kumar, M., & Philip, L. (2006b). Enrichment and isolation of endosulfan degrading mixed bacterial culture. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 41*(1), 81–96.

Kumar, M., & Philip, L. (2007). Biodegradation of endosulfan contaminated soil in a pilot scale reactor—bioaugmented with mixed bacterial culture. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 42*(6), 707–715.

Kumari, M., Ghosh, P., & Swati Thakur, I. S. (2014). Microcosmic study of endosulfan degradation by *Paenibacillus* sp ISTP10 and its toxicological evaluation using mammalian cell line. *International Biodeterioration Biodegradation, 96*, 33–40.

Kwon, G., Kim, J., Kim, T., Sohn, H., Koh, S., Shin, K., et al. (2002). *Klebsiella pneumoniae* KE-1 degrades endosulfan without formation of the toxic metabolite, Endosulfan sulfate. *FEMS Microbiology Letters, 215*, 255–259.

Kwon, J. S., Sohn, H. Y., Shin, K. S., Kim, E., & Seo, B. I. (2005). Biodegradation of the organochlorine insecticide, endosulfan, and the toxic metabolite, endosulfan sulfate, by *Klebsiella oxytoca* KE-8. *Applied Microbiology and Biotechnology, 67*, 845–850.

Lee, S. E., Kim, J. S., Kennedy, I. R., Park, J. W., Kwon, G. S., Koh, S. C., et al. (2003). Biotransformation of an organochlorine insecticide, endosulfan, by *Anabaena* species. *Journal of Agriculture and Food Chemistry, 51*, 1336–1340.

Lee, M. D., Thomas, J. M., Borden, R. C., Bedient, P. B., & Wilson, J. T. (1988). Biorestoration of aquifers contaminated with organic compounds. *Critical Reviews in Environmental Control, 18*, 29–89.

Lee, M. D., & Ward, C. H. (1985). Environmental and biological methods for the restoration of contaminated aquifers. *Environmental Toxicology and Chemistry, 4*, 743–750.

Li, W., Dai, Y., Xue, B., Li, Y., Peng, X., Zhang, J., et al. (2009). Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5. *Journal Hazard Materials, 167*, 209–216.

Li, Y. F., & Macdonald, R. W. (2005). Sources and pathways of selected organochlorine pesticides to the Arctic and effect of pathway divergence on HCH trends in biota: A review. *Science of the Total Environment, 342*, 853–858.

Madsen, E. L. (1991). Determining in situ biodegradation: Facts and challenges. *Environmental Science and Technology, 25*, 1663–1673.

Mansingh, A., & Wilson, A. (1995). Insecticide contamination of Jamaican Environment. Baseline studies on the status of Kingston Harbour. *Marine Pollution Bulletin, 30*, 640–645.

Martens, R. (1976). Degradation of (8-9 ^{14}C) endosulfan by soil microorganisms. *Applied and Environment Microbiology, 31*, 853–858.

Miles, J. R. W., & Moy, P. (1979). Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bulletin of Environment Contamination and Toxicology, 23*, 13–19.

Miles, C. J., & Pfeuffer, R. J. (1997). Pesticides in canals of south Florida. *Archives of Environmental Contamination and Toxicology, 32*, 337–345.

Monirith, I., Nakata, H., Tanaba, S., & Tana, T. S. (1999). Persistent organochlorine residues in marine and freshwater fish in Cambodia. *Marine Pollution Bulletin, 38*, 6045–6052.

Mukherjee, I., & Gopal, M. (1994). Degradation of beta-endosulfan by *Aspergillus niger*. *Toxicological and Environmental Chemistry, 46*, 217–221.

Narkhede, C. P., Patil, A. R., Koli, S., Suryawanshi, R., Wagh, N. D., Salunke, B. K., et al. (2015). Studies on endosulfan degradation by local isolate *Pseudomonas aeroginosa*. *Biocatalysis Agricultural Biotechnology, 4*(2), 259–265.

Penuela, G. A., & Barcelo, D. (1998). Application of C-18 disks followed by gas chromatography techniques to degradation kinetics, stability and monitoring of endosulfan in water. *Journal Chromatography A, 795*, 93–104.

Ressler, B. P., Kneifel, H., & Winter, J. (1999). Bioavailability of polycyclic aromatic hydrocarbons and formation of humic acid-like residues during bacterial PAH degradation. *Applied Microbiology and Biotechnology, 53*, 85–91.

Riser-Roberts, E. (2002). *Remediation of petroleum contaminated soils; biological, physical and chemical processes*. New York: Lewis Pub.

Sethunathan, N., Megharaj, M., Chen, Z., Singh, N., Kookana, R. S., & Naidu, R. (2002). Persistence of endosulfan and endosulfan sulfate in soil as affected by moisture regime and organic matter addition. *Bulletin of Environment Contamination and Toxicology, 68*, 725–731.

Sethunathan, N., Megharaj, M., Chen, Z. L., Williams, B. D., Lewis, G., & Naidu, R. (2004). Algal degradation of a known endocrine disrupting insecticide, alpha-endosulfan and its metabolites, endosulfan sulfate, in liquid medium and soil. *Journal of Agriculture and Food Chemistry, 52*, 3030–3035.

Shetty, P. K., Mitra, J., Murthy, N. B. K., Namitha, K. K., Savitha, K. N., & Raghu, K. (2000). Biodegradation of cyclodiene insecticide endosulfan by *mucor-thermo-hyalospora* MTCC 1384. *Current Science, 79*, 1381–1383.

Shivaramaiah, H. M., Sanchez-Bayo, F., Al-Rifai, J., & Kennedy, I. R. (2005). The fate of endosulfan in water. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 40*(5), 711–720.

Siddique, T., Okeke, B. C., Arshad, A., & Frankenberger, W. T. (2003). Enrichment and Isolation of endosulfan-Degrading Microorganisms. *Journal of Environmental Quality, 32*, 47–54.

Sims, R., & Bass, J. (1984). *Review of place treatment techniques for contaminated surface soils*, Vol 1. Technical evaluation. EPA report No. EPA-540/2-84-003a.

Singh, S., Dureja, P., Kumar, S., & Jain, M. C. (1999). Persistence of alpha and beta isomers of endosulfan and endosulfan sulfate in diverse soils on India as influenced by flooding. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 34*, 965–974.

Stewart, D. K. R., & Cairns, K. G. (1974). Endosulfan persistence in soil and uptake by potato tubers. *Journal of Agriculture and Food Chemistry, 22*, 984–986.

Sujatha, C. H., Nair, S. M., & Chacko, J. (1999). Determination and distribution of endosulfan and Malathion in an Indian estuary. *Water Research, 33*, 109–114.

Sunderam, R. I. M., Cheng, D. M. H., & Thompson, G. B. (1992). Toxicity of endosulfan to native and introduced fish in Australia. *Environmental Toxicology and Chemistry, 11*, 1469–1476.

Sutherland, T. D., Horne, I., Harcourt, R. L., Russel, R. J., & Oakeshott, J. G. (2002a). Gene cloning and molecular characterization of a two enzyme system catalyzing the oxidative detoxification of β-endosulfan. *Applied and Environment Microbiology, 68*, 6237–6245.

Sutherland, T. D., Horne, I., Harcourt, R. L., Russel, R. J., & Oakeshott, J. G. (2002b). Isolation and characterization of a *Mycobacterium* strain that metabolizes the insecticide endosulfan. *Journal of Applied Microbiology, 93*, 380–389.

Sutherland, T., Horne, I., Lacey, M., Harcourt, R., Russell, R., & Oakeshott, J. (2000). Enrichment of an endosulfan-degrading mixed bacterial culture. *Applied and Environment Microbiology, 66*, 2822–2828.

Sutherland, T. D., Weir, K. M., Lacey, M. J., Horne, I., Russel, R. J., & Oakeshott, J. G. (2002c). Enrichment of a microbial culture capable of degrading endosulphate, the toxic metabolite of endosulfan. *Journal of Applied Microbiology, 94*, 541–548.

Tanaba, S., Iwata, H., & Tatsukawa, R. (1994). Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Science of the Total Environment, 154*, 163–177.

Thangadurai, P., & Suresh, S. (2014). Biodegradation of endosulfan by soil bacterial cultures. *International Biodeterioration Biodegradation, 94*, 38–47.

Tiwari, M. K., & Guha, S. (2013). Kinetics of the biodegradation pathway of endosulfan in the aerobic and anaerobic environments. *Chemosphere, 93*, 567–573.

Topp, E., Tessier, L., & Gregorich, E. G. (1996). Dairy manure incorporation stimulates rapid atrazine mineralization in an agricultural soil. *Canadian Journal Soil Science, 76*, 403–409.

Verstraete, W., & Devliegher, W. (1996). Formation of non-bioavailable organic residues in soil: Perspectives for site remediation. *Biodegradation, 7*, 471–485.

Weber, J., Halsall, C. J., Muir, D., Teixeira, C., Small, J., Solomon, K., et al. (2010). Endosulfan, a global pesticide: A review of its fate in the environment and occurrence in the Arctic. *Science of the Total Environment, 408*, 2966–2984.

Worthing, C. R. (1983). *The pesticide manual. A worked compendium* (7th ed.). London: British Crop Protection Council.

Zhulidov, A. V., Headley, J. V., Pavlov, D. F., Roberts, R. D., Korotova, L. G., Vinnikov, Y. Y., et al. (2000). Riverline fluxes of the persistent organochlorine pesticides hexahlorocyclohexane and DDT in the Russian Federation. *Chemosphere, 41*, 829–841.

Bioremediation of Isoproturon Herbicide in Agricultural Soils

Sabir Hussain, Tanvir Shahzad, Muhammad Imran, Azeem Khalid and Muhammad Arshad

Abstract Isoproturon is a phenylurea herbicide widely used to control broad leaf weeds in cereal crops. It has been detected beyond the safe concentrations in a number of soil and water samples throughout the world. This review presents an overview of potential toxic effects of isoproturon and its fate in the environment. Moreover, major role of biodegradation as a permissible remedy tool in environmental decontamination to solve the problem of irrational use of this herbicide is highlighted. Recent advances in this area show that the microbial biodegradation of isoproturon can serve as a basis for the development of bioremediation processes in pure cultures and in the field.

Keywords Isoproturon · Toxicity · Biodegradation · Degradation pathways · Environmental factors

S. Hussain · T. Shahzad
Department of Environmental Sciences and Engineering,
Government College University, Faisalabad, Pakistan

M. Imran
Department of Soil Science, Muhammad Nawaz Shareef
University of Agriculture, Multan, Pakistan

A. Khalid (✉)
Department of Environmental Sciences, PMAS University of Arid Agriculture,
Rawalpindi, Pakistan
e-mail: azeem@uaar.edu.pk; azeemuaf@yahoo.com

M. Arshad
Institute of Soil and Environmental Sciences, University of Agriculture,
Faisalabad, Pakistan

M. Imran
Environmental Microbiology, Soil Science Division, Nuclear Institute
of Agriculture and Biology (NIAB), Faisalabad, Pakistan

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_4

1 Introduction

Agriculture is a key sector in the global economy where countries strive to improve crop productivity. Pesticides are widely used to increase crop production and limit production irregularities associated with crop pest infestation. No doubt, pesticide use ensures high yields of agricultural crops, but at the same time, it pollutes the soil and water resources. As a result of intensive and repetitive use, pesticide residues have increased manifolds and detected beyond the permissible limits in different compartments of the environment.

Isoproturon[3-(4-isopropylphenyl)-1, 1-dimethylurea] is a selective herbicide belonging to the phenyl urea family of herbicides. It is constituted by a phenyl ring (C_6H_4) with a C_1-branched methyl urea [(NH_2)-CO-NH-CH_3)] and C_4-branched dimethyl (Fig. 1). It is usually a colorless crystalline solid powder at room temperature (Table 1). It is highly stable over a wide range of pH (4–13), with the degradation half-life of at least more than 200 to 1560 days. It shows variable stability against the photolysis in water with a DT50 ranging from 4.5 to 88 days (AgriTox AFSSA-DIVE 2007). It is one of the most widely used herbicides in conventional agriculture in different regions of the world (Environment-Agency 2001; INERIS 2007; Yin et al. 2008). As a result of higher water solubility and intensive use, isoproturon has been detected in both surface and ground waters in Europe to the levels exceeding EU drinking water limit set at 0.1 $\mu g\ L^{-1}$ (Chhokar and Malik 2002; Muller et al. 2002; Sorensen et al. 2003). Earlier, it was included in the list of hazardous substances compiled by European Commission (European Commission 2001).

Isoproturon is one of the most commonly used herbicide for annual grasses, including bent grass, field foxtail, annual bluegrass, ryegrass, wild oats and several annual broadleaf weeds, such as feverfew, chickweed and the lady's mantle (RigaudJP and Lebreton 2004). It is absorbed by the weeds through their root system and transported to leaves. After the root penetration, isoproturon is translocated to aerial parts through xylem and causes symptoms of toxicity. It binds to D1 protein of the thylakoid membrane, inhibiting the electron transfer to the

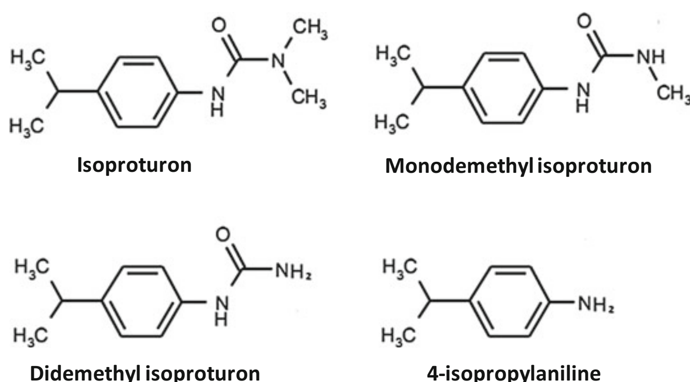


Fig. 1 Structural formulas of isoproturon and its metabolites

Table 1 Structure and physicochemical properties of isoproturon

Identification code	Isoproturon	
IUPAC name	3-(4-isopropylphenyl)-1,1-dimethylurea	AgriTox AFSSA-DIVE (2007)
CAS number	34123-59-6	AgriTox AFSSA-DIVE (2007)
Empirical formula	$C_{12}H_{18}N_2O$	Vrielynck et al. (2006)
Formula weight	206.28	Vrielynck et al. (2006)
Physical state	White crystalline	AgriTox AFSSA-DIVE (2007)
Melting point	152.4 °C	AgriTox AFSSA-DIVE (2007)
Decomposition temperature	239 °C	Yu et al. (2003)
Molar melting enthalpy (ΔHm)	21.33 J K^{-1} mol^{-1}	Yu et al. (2003)
Entropy (ΔSm)	50.13 J K^{-1} mol^{-1}	Yu et al. (2003)
Water solubility at 20 °C	70 mg L^{-1}	AgriTox AFSSA-DIVE (2007)
Vapour pressure at 20 °C	3.3 μPa	AgriTox AFSSA-DIVE (2007)
Henry's law constant	14.6 e^{-6} Pa m^3 mol^{-1}	AgriTox AFSSA-DIVE (2007)
Temperature	293(2) K	Vrielynck et al. (2006)
Wavelength	0.71073 Å	Vrielynck et al. (2006)
Crystal system	Orthorhombic	Vrielynck et al. (2006)
Volume	2357.4(7) $Å^3$	Vrielynck et al. (2006)
Z	8	Vrielynck et al. (2006)
Density	1.162 mg m^{-3}	Vrielynck et al. (2006)
Absorption coefficient	0.075 mm^{-1}	Vrielynck et al. (2006)
Extinction coefficient	0.0011(6)	Vrielynck et al. (2006)

photosystem II (PSII) and thus, induces oxidative stress. As a result, lipids, proteins and other cellular components are damaged, as evidenced by necrosis and chlorosis (Caux et al. 1998; Yin et al. 2008). Besides inhibition of growth, it produces some toxic compounds which cause cell death (Mittler 2002; Hassan and Nemat-Alla 2005). The ecotoxicological data have suggested that isoproturon and a few of its main metabolites are carcinogenic in nature and hence harmful to humans, animals, plants, aquatic invertebrates, fresh water algae and microbial activities (Orton et al. 2009; Vallotton et al. 2009; Dosnon-Olette et al. 2010, 2011). So, there is a dire need to understand the processes involved in the degradation of isoproturon in agricultural soils by developing efficient and practical removal techniques and to prevent its dispersion in the environment. The degradation of isoproturon primarily occurs through demethylation leading to the formation of two main metabolites, such as monodemethyl isoproturon (MDIPU) and didemethyl isoproturon (DDIPU). Besides, phenylurea side chain is hydrolyzed into 4-Isopropylaniline (Hussain et al. 2009).

In this chapter, we have reviewed the current state of the art in ecotoxicology and fate of isoproturon after its entry into the soil environment. Besides, biotic and abiotic processes for the transformation of isoproturon and factors affecting its fate in the environment have been thoroughly discussed.

2 Ecotoxicology of Isoproturon

Ecotoxicological data as shown in Table 2 based on several published studies have suggested that isoproturon and some of its major metabolites are very harmful to animals, plants, microorganisms and human beings (Dosnon-Olette et al. 2010, 2011; Knauert et al. 2010; Paris-Palacios et al. 2010). Isoproturon was found to have dose-dependent mutagenic effect, resulting in chromosomal aberration and sperm shape abnormality (Behera and Bhunya 1990). Similarly, it causes potential hepatocarcinogenicity in rats and also increases probability of tumor formation (Hoshiya et al. 1993). Orton et al. (2009) reported that isoproturon had an endocrine disruption potential, affect ingantiesterogenic activity, antiandrogenic activity, ovulation and testosterone production. In addition, aniline metabolite of isoproturon, 4-IA was found to be 600 times more toxic than parent compound isoproturon (Tixier et al. 2002).

Isoproturon, applied on soil microcosms, exerts a selection pressure on the soil algal community, causing on elimination of sensitive diatom species, but favouring the development of more resistant periphytons (Pérés et al. 1996). The results of assay regarding inhibition of the algal growth upon exposure to isoproturon showed EC_{50} (72 h) of 0.077 mg L^{-1} for *Scenedesmus subspicatus* and EC_{50} (7 days) of 0.06 mg L^{-1} for *Lemna minor* (Nitschke et al. 1999). Further studies indicated that isoproturon adversely affected the algal growth and its metabolism. Upon exposure to isoproturon (10 μg L^{-1}) for 4 days, growth rates and photosynthetic activities of the algal strains *Scenedesmus obliquus* and *Scenedesmus quadricauda* were considerably inhibited (Dosnon-Olette et al. 2010). The growth and effective quantum yields of the algal strain *Scenedesmus vacuolatus* were also found to be reversibly inhibited depending uponthe concentration and exposure times of isoproturon (Vallotton et al. 2009). Schmitt-Jansen and Altenburger (2005) studied the toxic

Table 2 Nomenclature of isoproturon and its known metabolites

Current name	IUPA name	Molecular formula	Molecular weight	Abbreviation
Isoproturon	3-(4-isopropylphenyl)-1, 1-dimethylurea	$C_{12}H_{18}N_2O$	206.29 g mol^{-1}	IPU
Monodemethyl lisoproturon	3-(4-isopropylphenyl)-1-methylurea	$C_{11}H_{16}N_2O$	192 g mol^{-1}	MDIPU
Didemethyl isoproturon	3-(4-isopropylphenyl)-urea	$C_{10}H_{14}N_2O$	178 g mol^{-1}	DDIPU
4-isopropylaniline	4-isopropyl-aniline	$C_9H_{13}N$	135 g mol^{-1}	4-IA

effects of isoproturon on periphyton communities in a microcosm environment and found that the biomass production of the algal community was decreased in response to increasing concentration of isoproturon. It was also concluded that isoproturon can modify structure of algal community even at sub-acute toxicity level.

Besides the targeted weeds, isoproturon has shown pronounced effects on non-target plants, including macrophytes and agricultural crops. The germination and growth of wheat (*Triticum aestivum*) and beans were drastically inhibited by isoproturon (Yin et al. 2008; Liang et al. 2012). Furthermore, isoproturon modified the activity of antioxidant enzymes and chromosomal mitotic activity of wheat and reduced the chlorophyll content even at low concentration (2 mg kg^{-1}) (Nemat-Alla and Hassan 2007; Yin et al. 2008; Kumar 2010). Likewise, isoproturon was found to cause toxic effect on phytoplanktons (Knauert et al. 2008; Dosnon-Olette et al. 2011). Photosynthetic efficiency of three submerged macrophytes, such as *Elodea canadensis*, *Myriophyllum spicatum* and *Potamogeton lucens*, was drastically inhibited, following their exposure to isoproturon (Knauert et al. 2010). Also, isoproturon can enter the food chain by the process of bioaccumulation in non-target plants. Such non-target bioaccumulation was observed in macrophyte *Lemna minor* (Bottcher and Schroll 2007).

Isoproturon can also affect animal health. Daily injection of 675 mg kg^{-1} of isoproturon in rats significantly increased peroxidation reactions in cerebral tissues (Hazarika and Sarkar 2001). The peroxidation generates the appearance of reactive forms of oxygen species which alter the cellular membrane and to some extent, it can trigger cell death. Isoproturon was also detected in the water earthworm *Tubifex tubifex* (Paris-Palacios et al. 2010). An increase in the concentration of isoproturon (0.1–10 mg L^{-1}) and the exposure time (4–7 days) resulted in an increase in 5–37 % mortality of *Tubifex tubifex*. Although isoproturon showed lethal effects on earthworms, including *Tubifex*, but sub-lethal effects, such as reduced growth rate, decreased protein content and increased activity of antioxidant defence enzymes were also observed (Mosleh et al. 2005; Mosleh 2009). The use of isoproturon in agricultural soil also negatively affected the development of early larval stages of spawn and tadpoles of amphibians (Greulich et al. 2002).

Isoproturon is also known to affect microbial activity and community composition, including bacteria and fungi (Widenfalk et al. 2004, 2008). A significant effect of isoproturon on the lower level of their community organization and insignificant effect on community level have been reported (Widenfalk et al. 2008).

3 Fate of Isoproturon in the Environment

There are several routes by which isoproturon can contaminate the environment. After its application to agricultural ecosystems, dispersion of isoproturon from the field to different compartments of the surrounding agro-ecosystem is affected by various processes, including plant uptake, transfer through leaching or run-off, adsorption and abiotic and biotic degradation. Following its application, adsorption

and desorption on the soil components mainly determine the fate and dynamics of isoproturon. Compared to other herbicides, isoproturon adsorption is relatively weak and comparable to that of atrazine, but much inferior to that of other phenyl urea herbicides (Perrin-Ganier et al. 1995). Isoproturon had a K_d value below 10 L kg^{-1} which suggests that isoproturon is poorly retained in the soils (Tian et al. 2010). The K_d of isoproturon is dependent on organic carbon content in the surface soil (El-khattabi et al. 2007; Tian et al. 2010) while it is dependent on clay content in sub-surface soil (Coquet et al. 2004). pH is also an important factor to determine the extent of adsorption of isoproturon. In general, it is adsorbed more in a solution of pH 5 than pH 8 (Ertli et al. 2004). The adsorption of isoproturon decreased with an increase in temperature (Amita et al. 2005). Similarly, its adsorption is also affected by agricultural practices (Si et al. 2006, 2011; Tian et al. 2010; El-Arfaoui et al. 2010). Recently, Eibisch et al. (2015) found that sorption of isoproturon in soil was altered not only by quality, but also by the quantity of pyrochars and hydrocharsin.

Isoproturon is transformed in the environment by the involvement of either physicochemical processes or biotic processes. Isoproturon transformation via chemical processes is very slow in most of the agricultural soils. Usually, it is stable in aqueous solution at pH between 4 and 10 and at moderate temperatures (Gerecke et al. 2002). However, under the influence of UV radiations, isoproturon can be photochemically decomposed in the water in upper surface of soil, leading to formation of demethylated metabolites (Parra et al. 2000; Rubio et al. 2006; Reddy et al. 2011). Isoproturon was reported to be degraded by Photo-Fenton photocatalytic process in aquatic systems under natural light (Rubio et al. 2006; Lopez-Munoz et al. 2013). Moreover, several studies have revealed photocatalytic degradation of isoproturon and its main metabolites by solar light in aqueous systems are also formed in the presence of the photo catalysts, such as TiO_2 (Sharma et al. 2008b, 2010; Reddy et al. 2010, 2011). However, this photocatlytic activity was further enhanced when TiO_2 was supported on different types of efficient supports including Al-MCM-41 (Sharma et al. 2008a), HY (Sharma et al. 2008b), H-Mordenite (Sharma et al. 2008a), zeolite (Reddy et al. 2012), porous nanosilica (Sharma et al. 2009) and SBA-15 (Sharma et al. 2008c). Similarly, isoproturon photocatalytic activity was also found to be enhanced when TiO_2 was doped with C, N and S by hydrolysis process (Reddy et al. 2010). The solarization by polyethylene mulching also promoted the transformation of isoproturon in agricultural soils (Navarro et al. 2009). Moreover, ozonation induces the hydroxylation of aromatic ring or side chain and can break the isopropyl alkyl chain (Mascolo et al. 2001). Isoproturon undergoes alkaline hydrolysis both in the presence and absence of sodium lauryl sulfate (NaLS) and cetyltrimethyl ammonium bromide (CTAB) (Gangwar and Rafiquee 2007a, b). The rate of alkaline hydrolysis is enhanced by CTAB but inhibited by NaLS.

Despite of the fact that isoproturon is transformed by a number of physicochemical processes, the transformation by living systems is considered as a dominant phenomenon in the agricultural soils. Early studies have indicated that isoproturon is degraded slowly by microorganisms (Larsen et al. 2000; Kristensen et al. 2001; Sorensen et al. 2001). Later studies showed that agricultural soils, that are regularly

exposed to isoproturon, had a greater degradation ability than un-exposed soils (Reid et al. 2005; Hussain et al. 2011, 2013). Reid et al. (2005) found isoproturon catabolic activity significantly higher in soils treated with this herbicide than untreated soil. El-Sebai et al. (2005, 2007) reported enhanced isoproturon biodegradation in a French agricultural field of "Le Souiche" repeatedly treated with this herbicide. Similarly, Hussain et al. (2011, 2013) investigated the mineralization of isoproturon in a French agricultural field of "Epoisses" over a three year crop rotation in relation to physic-chemical properties of the soil and pesticide application history. They observed a strong relationship between mineralization of isoproturon and application history of the pesticide as well as physicochemical properties of the soil. The isoproturon mineralization activity was not found to be evenly distributed across agricultural fields. Beck et al. (1996) reported that the time for 50 % dissipation of isoproturon ranged from 31 to 483 days in 25 different samples collected from the same agricultural field. This observation highlighted the importance of spatial heterogeneity of isoproturon degrading activity at the field scale. After the initial report by Beck et al. (1996), spatial variability in isoproturon degradation was reported by a number of scientists working in different countries like Denmark, UK and France (Bending et al. 2006; Rodriguez-Cruz et al. 2006; El-Sebai et al. 2007, 2011; Vieublé-Gonod et al. 2009; Hussain et al. 2013). However, the spatial variability was found to be associated with variation in different pedoclimatic factors, such as pH, microbial biomass, organic matter content, moisture, agricultural practices and pesticide application history. Spatial heterogeneity of microbial activity responsible for degradation of isoproturon in agricultural fields encouraged scientists to conduct further studies to identify underlying processes and mechanisms for isoproturon biodegradation in different parts of the world.

4 Biodegradation of Isoproturon

Biodegradation of isoproturon is considered to be the main phenomenon responsible for its natural attenuation from the environment. Hence, a number of researchers have focused their attention on investigating the biodegradation of isoproturon by living organisms. It was observed that microalgae belonging to genus *Scenedesmus* removed 58 % of the initially added isoproturon in the liquid culture (Dosnon-Olette et al. 2010). Low concentration (10 μg L^{-1}) of isoproturonwas removed up to 25 % by an aquatic plant *Lemna minor* (Dosnon-Olette et al. 2011). Based on these findings, it was suggested that although the growth of *Lemna minor* was affected by higher concentrations of isoproturon, but it can be used to phytoremediate isoproturon at lower concentrations. However, isoproturon metabolism resulted in an accumulation of its metabolites monodemethylisoproturon (MDIPU), didemethylisoproturon (DDIPU) and 4-IA in some earthworms like *Tubifex* (Paris-Palacios et al. 2010) and even in some amphibians spawn and tadpoles (Greulich et al. 2002). However, a number of previous and recent studies indicate that microbial biodegradation is the primary mechanism of isoproturon

degradation and dissipation in the soil environment (El-Sebai et al. 2007; Hussain et al. 2011, 2013). This phenomenon stimulated the research aiming at isolating and characterizing microbial strains which are able to entirely mineralize isoproturon from the contaminated soils.

4.1 Biodegradation of Isoproturon by Bacteria

To-date, several bacterial species capable of degrading isoproturon have been reported (Table 3), however, most of the isolated bacterial strains partially degraded this pesticide (Cullington and Walker 1999; Turnbull et al. 2001; Tixier et al. 2002; Dwivedi et al. 2011). However, some species belonging to genus *Sphingomonas*

Table 3 Isoproturon degrading fungal and bacterial strains

Strain	Comments	References
Microbial strains		
Arthrobacter globiformis D47	This bacterial strain had the potential to degrade isoproturon and other structurally related phenylurea herbicides including diuron, linuron, monolinuron and metoxuron into their aniline derivatives	Cullington and Walker (1999)
Sphingomonas sp. SRS2	This strain had the potential to mineralize isoproturon. It could also degrade other dimethylurea substituted hebicidesdiuron and chlorotoluron	Sorensen et al. (2001)
Arthrobacter sp. N2	This bacterial strain had the potential to degrade isoproturon and other structurally related phenylurea herbicides including diuron and chlorotoluron into their aniline derivatives	Tixier et al. (2002)
Sphingomonas sp. F35	This strain had the potential for the biodegradation of isoproturon in agricultural soil having neutral to slightly alkaline pH	Bending et al. (2003)
Methylopila sp. TES	This strain could mineralize isoproturon and its metabolites. However, it could not degrade other structurally related phenylurea herbicides	El-Sebai et al. (2004)
Sphingobium sp. YBL1	This strain had the potential to mineralize isoproturon. It could also degrade other structurally related phenylurea herbicides such as diuron, chlorotoluron and fluometuron	Sun et al. (2009)
Sphingobium sp. YBL2	This strain had the potential to mineralize isoproturon. It could also degrade other structurally related phenylurea herbicides such as diuron, chlorotoluron and fluometuron	Sun et al. (2009)
Sphingobium sp. YBL3	This strain had the potential to mineralize isoproturon. It could also degrade other structurally related phenylurea herbicides such as diuron, chlorotoluron and fluometuron	Sun et al. (2009)
Pseudomonas aeruginosa JS-11	This strain had the potential for partial biodegradation of isoproturon along with plant growth promoting and biocontrol activities	Dwivedi et al. (2011)

(continued)

Table 3 (continued)

Strain	Comments	References
Sphingomonas sp. SH	This strain had the potential to mineralize isoproturon. However, it could not degrade other structurally related phenylurea herbicides	Hussain et al. (2011)
Fungal strains		
Rhizoctonia sp.	This strain had the potential to degrade isoproturon	Steiman et al. (1994)
Rhizoctonia solani	This fungal strain had the potential to degrade isoproturon, diuron and chlorotoluron without accumulation of any metabolite	Vroumsia et al. (1996)
Bjerkandera adusta	This fungal strain had the potential to degrade isoproturon, diuron and chlorotoluron	Khadrani et al. (1999)
Oxysporus sp.	This fungal strain had the potential to degrade isoproturon, diuron and chlorotoluron	Khadrani et al. (1999)
Cunninghamella elegans JS/2	This fungal strain had the potential to degrade isoproturon into hydroxylated metabolites	Hangler et al. (2007)
Mortierella sp. Gr4	This strain could degrade isoproturon, diuron, linuron and chlorotoluron through demethylation. However, it could also hydroxylate isoproturon	Badawi et al. (2009)

and *Methylopila* showed their ability to degrade isoproturon completely (Sorensen et al. 2001; El-Sebai et al. 2004; Sun et al. 2009; Hussain et al. 2011).

Sorensen et al. (2001) isolated the first isoproturon mineralizing bacterial strain, *Sphingomonas* sp. SRS2, from the Deep Slade agricultural soil of UK. This bacterial strain had the ability to degrade not only the known isoproturon metabolites (MDIPU, DDIPU and 4-IA), but also some closely related phenylurea herbicides like diuron and chlorotoluron. Based on the transient accumulation of metabolites during isoproturon metabolism, the initial steps of isoproturon metabolic pathway were proposed for *Sphingomonas* sp. SRS2 (Sorensen et al. 2001, 2003). Further research indicated that isoproturon mineralizing activity of the strain SRS2 was significantly enhanced when it is co-cultured with an unidentified bacterial strain SRS1 or in the presence of certain amino acids in the liquid medium (Sorensen et al. 2002). Shi and Bending (2007) reported that isoproturon biodegradation was associated with the changes in the structure of *Sphingomonas* spp. communities including the proliferation of isoproturon mineralizing *Sphingomonas* sp. SRS2 in the soil. In order to further elucidate the role of *Sphingomonas* sp. SRS2, Kristensen et al. (2006) carried out genetic labeling of this strain with mini-Tn5-*lux*AB marker to use it as a biomarker to evaluate the bioremediation potential of this strain. Another isoproturon mineralizing bacterial strain *Methylopila* sp. TES was isolated and characterized from an isoproturon treated agricultural field of France (El-Sebai et al. 2004). This bacterial strain had the ability to degrade isoproturon as well as known metabolites, however, this strain could not degrade other structurally related phenylurea herbicides. Based on these findings, it was hypothesized that *Methylopila* sp. TES probably had a catabolic pathway which is highly specific for

isoproturon degradation. Similar findings have also been reported by Hussain et al. (2009, 2011) while studying the mineralization of isoproturon by *Sphingomonas* sp. SH and enriched mixed bacterial culture isolated from different French agricultural soils. Sun et al. (2009) described the isolation and characterization of three isoproturon mineralizing *Sphingobium* strains YBL1, YBL2 and YBL3 from the soils of two herbicide manufacturing plants. The isoproturon degradation by these bacterial strains was found to be initiated by N-demethylation of the urea side chain, resulting in accumulation of first metabolite MDIPU. These three strains had also the ability to degrade related dimethylurea substituted herbicides, such as chlorotoluron, diuron and fluometuron. Strain YBL1 was also capable of degrading methoxymethylphenyl-urea herbicides, such as linuron. Furthermore, accumulation of catechol and the first metabolite after phenyl ring cleavage i.e. cis, cis-muconic acid, was also observed during the mineralization of aniline by *Sphingobium* sp. strain YBL2 which suggested β-ketoadipate pathway for degradation. This hypothesis was further reinforced by the amplification of *catA* gene coding for catechol 1, 2-dioxygenase from isoproturon mineralizing *Sphingobium* sp. strains YBL2 (Sun et al. 2009) and *Sphingomonas* sp. SH (Hussain et al. 2011). Hussain et al. (2009) reported involvement of a mixed bacterial culture dominated by *Sphingomonas* spp. (>90 %) in the mineralization of isoproturon. Thus, it was clearly evident that isoproturon mineralization activity was associated with the bacterial isolates predominantly belonging to family Sphingomonadaceae including *Sphingobium* sp. (Sun et al. 2009) and *Sphingomonas* sp. (Sorensen et al. 2001, 2002; Bending et al. 2003; Hussain et al. 2009, 2011). This implies that members of this genus possess broad catabolic activities (White et al. 1996; Fredrickson et al. 1999; Sorensen et al. 2001), and they can rapidly cause isoproturonmineralization to use it as a source of carbon and nitrogen.

Roberts et al. (1998) isolated several bacterial strains capable of using isoproturon as the source of carbon and energy. They found that some of these strains were also able to degrade two main metabolites of isoproturon (MDIPU and DDIPU) as well as other phenyl urea herbicides, like diuron and linuron. The transient accumulation of MDIPU and DDIPU was observed during the biodegradation of isoproturon. Cullington and Walker (1999) isolated a bacterial strain *Arthrobacter globiformis* which was able to use diuron as the sole source of carbon and nitrogen. *Arthrobacter globiformis* also had the ability to slowly transform other phenylurea herbicides including isoproturon to their aniline derivatives by the hydrolysis of urea side chain. They showed that the degradation performance of *A. globiformis* could be further improved by providing additional carbon and energy sources. Further characterization of this bacterial strain led to identification of a catabolic plasmid harboring a phenylurea hydrolase gene *puhA* (Turnbull et al. 2001). Later on, similar type of isoproturon catabolic activity was also reported in *Arthrobacter* sp. N2 (Tixier et al. 2002). Dwivedi et al. (2011) reported the isolation and characterization of a *Pseudomonas aeruginosa* strain JS-11 from the wheat rhizosphere. In addition to isoproturon degradation, this bacterial strain had also the ability to function as plant growth promoter and as an agent for disease management.

4.2 Biodegradation of Isoproturon by Fungi

Although most studies have focused on bacteria, yet a number of studies also suggest the involvement of fungi in the degradation of isoproturon (Table 3). Vroumsia et al. (1996) reported that strains belonging to *Zygomycetes*, *Agonomycetes* and *Rhizoctonia* have the ability to degrade isoproturon. However, the strains of genus *Rhizoctonia* were found more efficient to degrade 84 % of the initially added isoproturon within two days than other two. Moreover, no accumulation of metabolites of the herbicide was observed. These results also confirm the results of Steiman et al. (1994) who reported that *Rhizoctonia* sp. had the ability to rapidly degrade isoproturon. Some fungal strains of the genus *Basidiomycetes*, *Ascomycetes* and *Zygomycetes* were also able to transform isoproturon to hydroxylated or demethylated products. Hydroxylation was found to be a dominant mode of transformation by these fungi (Ronhede et al. 2005). Interestingly, the mineralization of isoproturon in the soil was found to be stimulated by the hydroxylation process (Ronhede et al. 2007). Similarly, Hangler et al. (2007) reported that *Cunninghamella elegans* JS/2 was able to convert isoproturon into several metabolites. The degradability of this strain was inducible in response to isoproturon exposure and that several inducible enzymes were involved in the degradation of isoproturon. Badawi et al. (2009) reported that a soil fungus *Mortierella* sp. Gr4 had the ability to degrade isoproturon by two successive processes, namely N-demethylation of the urea side chain and hydroxylation of isopropyl ring constituents. This study also suggests that the fungal pathways for degradation of isoproturon herbicides differ apparently from the pathways that are followed by the bacterial strains.

5 Metabolic Pathways Involved in the Biodegradation of Isoproturon

Degradation of isoproturon in soil environment primarily takes place through microbial processes (Ronhede et al. 2005). Although both bacteria and fungi can degrade isoproturon, but degradation pathways are mostly derived from the bacterial species. However, the metabolic pathway for complete removal of isoproturon is not yet clearly elucidated, although steps for the initial transformation of isoproturon have been proposed by several investigators (Sorensen et al. 2001, 2003; Badawi et al. 2009; Sun et al. 2009). Based on the formation and detection of isoproturon metabolites in a series of studies, Hussain et al. (2015) recently proposed a plausible metabolic pathway for the transformation of isoproturon (Fig. 2). According to the proposed pathway, demethylation of dimethyl urea side chain of isoproturon results in the formation of MDIPU. This initial step is often recognized as the limiting step of the isoproturon degradation. MDIPU is not only found in bacterial and fungal cultures, but also in high concentrations detected in agricultural

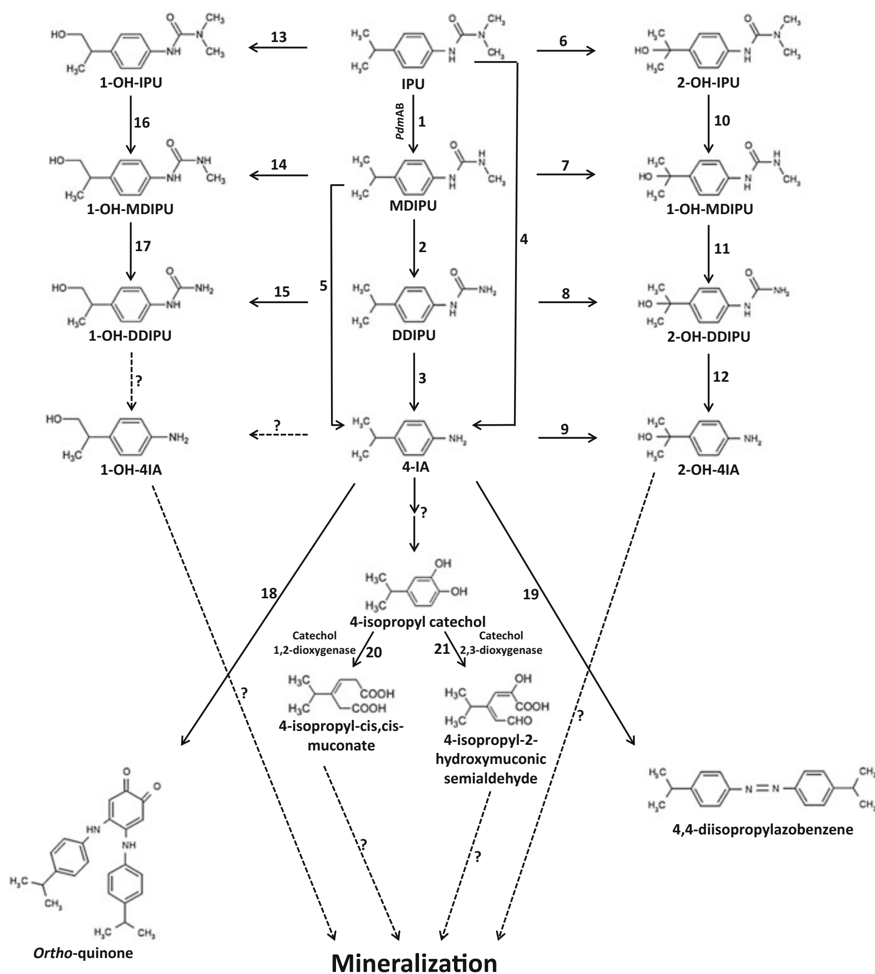


Fig. 2 Metabolic pathways of isoproturon proposed by Hussain et al. (2015)

soils after degradation of isoproturon (Berger 1999; Perrin-Ganier et al. 2001; Badawi et al. 2009; Hussain et al. 2011). Based on metabolites formed during the transformation of isoproturon by *Sphingomonas* sp. SRS2 and an enriched bacterial culture (Sorensen et al. 2001, 2003; Hussain et al. 2009), a metabolic pathway has been proposed which is initiated by two successive N-demethylations of isoproturon to MDIPU and DDIPU, followed by the cleavage of urea side chain to 4-IA and then mineralization of phenyl structure. In a fungal culture, successive demethylations of isoproturon resulted in the formation of MDIPU and DDIPU (Badawi et al. 2009). Recently, the molecular basis for this initial demethylation in *Sphingobium* sp. YBL2 was elucidated by Gu et al. (2013). They suggested that this demethylation was catalyzed by *Pdm*AB demethylase genes belonging to the

Rieske non-heme iron oxygenase (RO) family harbored by an isoproturon mineralizing bacterial strain *Sphuingobium* sp. YBL2. Furthermore, these genes were found to be organized in a transposable element and are highly conserved in other phenylurea herbicide degrading bacterial strain *Sphingomonas* sp. SRS2 which was isolated from agricultural soil in the UK. This explains why a common initial metabolic step is required during the degradation of phenylurea herbicides. Furthermore, *Arthrobacter globiformis* D47 (Turnbull et al. 2001) and *Arthrobacter globiformis* N_2 (Tixier et al. 2002) were found to degrade isoproturon directly into 4-IA by hydrolytic cleavage of the urea side chain without forming MDIPU and DDIPU. Two alternative metabolic pathways were also proposed based on the hydroxylation of urea side chain of IPU resulting in 1-OH-IPU or 2-OH-IPU in fungal strains, mixed bacterial cultures and in agricultural soils (Sorensen et al. 2003; Badawi et al. 2009; Maqbool et al. 2016). However, very little information is available on these hydroxylated metabolites.

Aniline compounds are generally metabolized through formation of a key intermediate metabolite catechol by their oxidative deamination (Parris 1980; Sorensen et al. 2003). Catechol is further degraded by two different ring cleavage pathways, like *ortho*-cleavage and *meta*-cleavage (Sun et al. 2009; Zhang et al. 2012). Catechol is a key intermediate of the β-ketoadipate pathway responsible for degradation of many aromatic compounds (Alva and Peyton 2003; Nayak et al. 2009) to corresponding aliphatic metabolite i.e. cis, cis-muconic acid (Nayak et al. 2009). This key intermediate was also detected during the mineralization of aniline by the isoproturon degrading bacterial strains YBL1, YBL2 and YBL3 (Sun et al. 2009). In addition, *catA* gene coding for catechol 1, 2-dioxygenase was detected in *Sphingobium* strains YBL1, YBL2 and YBL3 (Sun et al. 2009) and *Sphingomonas* sp. SH (Hussain et al. 2011), suggesting that phenyl ring of isoproturonis most likely degraded through *ortho*-cleavage of catechol. Recently, catechol-2, 3-dioxygenase activity was also found to be induced in the presence of isoproturon, indicating that aromatic ring cleavage might take place through *meta*-cleavage, resulting into the conversion of catechol to 4-isopropyl-hydroxymuconic semialdehyde (Zhang et al. 2012). However, this finding requires further elucidation. Aniline compounds, including 4-IA, undergo various chemical reactions in soils to form various types of products (Pieuchot et al. 1996; Scheunert and Reuter 2000; Perrin-Ganier et al. 2001). The 4-IA also forms dead-end products, such as disubstituted *ortho*-quinone and 4, 4-diisopropylazobenzene (Scheunert and Reuter 2000; Perrin-Ganier et al. 2001; Sorensen et al. 2003).

6 Factors Affecting Biodegradation of Isoproturon

The biodegradation of isoproturon is influenced by both biotic and abiotic factors in the soil. Among physicochemical factors, pH is one of the most important factors that have a great impact on the degradation of pesticide by microorganisms. There are a number of studies showing a relationship between pH and isoproturon

biodegradation not only in the soil (El-Sebai et al. 2011; Hussain et al. 2013), but also in pure cultures (Bending et al. 2003; Hussain et al. 2009, 2011; Sun et al. 2009). The effect of pH on isoproturon biodegradation was reported in a study conducted on 30 soil samples taken from different locations at the Brimstone farm experimental site at Oxfordshire, UK (Walker et al. 2002). The authors found a strong correlation between isoproturon degradation and soil pH. Similarly, accelerated degradation of isoproturon in these agricultural fields was found to be associated with the proliferation of isoproturon degrading microorganisms in relation to pH (Bending et al. 2003). Moreover, spatial variability of isoproturon mineralization (determined for 50 soil samples within an agricultural field in France) was also related to pH (El-Sebai et al. 2005, 2007). In addition to the field conditions, pH has also been reported to affect the isoproturon degrading activity in pure cultures (Hussain et al. 2009, 2011; Sun et al. 2009). In pure microbial cultures, a pH range of 7–7.5 was found to be optimum for the metabolism of isoproturon (Bending et al. 2003; Hussain et al. 2011).

Other soil and environmental factors, such as temperature, moisture, aeration and microbial biomass also affect the biodegradation of isoproturon (Issa and Wood 2005; Alletto et al. 2006; Schroll et al. 2006; Bending and Rodriguez-Cruz 2007; Grundmann et al. 2011; El-Sebai et al. 2011; Fenlon et al. 2011). The optimal temperature for the isoproturon mineralization in two different agricultural soils was found to be between 22 and 25.8 °C (Alletto et al. 2006; El-Sebai et al. 2011). However, 30 °C was optimum temperature for efficient degradation of isoproturon in broth culture (Sun et al. 2009). A huge variation in soil moisture content was observed for maximum biodegradation of isoproturon. For example, biodegradation of isoproturon was highest in a heterogeneous soil of France at soil moisture content of 90 % of water holding capacity (Issa and Wood 2005), while 24 % soil moisture was suitable for this herbicide biodegradation in another agricultural soil in France (El-Sebai et al. 2011). Moreover, degradation of isoproturon was higher in the surface soils than to sub-surface soils (Issa and Wood 2005; Bending and Rodriguez-Cruz 2007). Soil clay content as well as aging of isoproturon and its metabolites in agricultural soils affect isoproturon biodegradation by determining its sorption and availability in soils (Johannesen et al. 2003; Alletto et al. 2006). Different types of organic amendments including compost, sewage sludge and charcoal in the soils have been reported to affect the degradation of isoproturon by changing the sorption behavior in soil (Vieublé-Gonod et al. 2009; Si et al. 2011; Reid et al. 2013). Recently, amendment of soil with pyrochar and hydrochar has been found to increase the sorption of isoproturon in an agricultural soil, resulting into a decrease in its mineralization (Eibisch et al. 2014). Sun et al. (2009) reported that isoproturon degradation by the three bacterial strains was enhanced by addition of Mg^{+2} and significantly inhibited by addition of Ni^{+2} and Cu^{+2} in the broth cultures. The degradation of isoproturon in a wheat rhizosphere was enhanced in response to a treatment with salicylic acid (Lu et al. 2015). Hussain et al. (2013) mapped isoproturon mineralization in a French agricultural field over a three year crop rotation. They found that the activity and variability of isoproturon

mineralization was associated not only with different pedoclimatic factors and equivalent humidity, but also with pesticide application history over the previous three years.

In addition, the degradation rate of isoproturon was found to be linked to growth (Sorensen et al. 2003). Bending et al. (2001) reported that heterogeneity of isoproturon metabolic activity along the transect of soil was most likely due to variation in the dynamics of the isoproturon degrading microbial communities. One of the very important factors affecting the biodegradation is the repeated exposure of a soil to a pesticide treatment which often leads to the adaptation of soil microbial community for accelerated degradation (Racke and Coats 1990; El-Sebai et al. 2005). The potential of adaptation of a soil for the degradation of a pesticide can last for a few weeks to few years after the pesticide treatment (Walker and Welch 1991). This adaptation is considered as a dominant phenomenon affecting the biodegradation of pesticides in the soils (Soulas 1993; Cox et al. 1996; Sorensen et al. 2001; Sorensen and Aamand 2003). In fact, the repeated application of isoproturon in a target soil induces the establishment of isoproturon degrading microorganisms.

7 Conclusions and Future Perspectives

Due to the widespread use of isoproturon in agricultural fields, there is growing concern about the adverse effects of this herbicide on human and environment. Bioremediation can be an effective tool to harness microbial metabolic pathways for in situ and ex situ detoxification of isoproturon. Several studies have shown that bacteria and fungi can degrade isoproturon in both pure culture and soil, but the bacterial communities play a more dominant role in its mineralization. In particular, the role of bacterial strains belonging to the family *Sphingomonadaceae* in isoproturon mineralization is evidently proved. In order to clarify the involvement of the bacterial strains belonging to this family in isoproturon mineralization, there is need to plan further experiments aiming at isolation of efficient bacterial strains from different geological regions of the world. Moreover, there is also need to understand the genetics behind degradation process of isoproturon by bacterial trains. There may be the involvement of putative transcriptional, translational and post-translational degrading genes which has to be confirmed by identifying the genes involved in the biodegradation of isoproturon. Several studies indicate that the biodegradation of isoproturon was faster in the fields with a history of repeated application of this herbicide. The use of molecular markers may assist identification and tracking of the most potent microbial isolates with effective isoproturon degrading abilities. The induction of catabolic genes and expression of the desired herbicide-degrading enzyme(s) in the indigenous microbes can also increase their potential for use in bioremediation of contaminated agricultural fields.

Although initial steps involved in upper isoproturon metabolic pathways (isoproturon to 4-IA) have been identified, however, the lower metabolic pathways

(phenyl ring cleavage after 4-IA) and genes involved in the whole isoproturon metabolism are still unknown. Based on amplification of *cat*A gene in some isoproturon mineralizing bacterial strains, it is hypothesized that IPU phenyl ring cleavage during the mineralization is routed through catechol formation. There is a need to confirm whether mineralization of isoproturonoccurs by the accumulation of catechol as an intermediate metabolite. Further research should be conducted to identify the metabolites formed during the mineralization of isoproturon using more sensitive detection methods, such as GC-MS and UPLC. Catechol formation could be critical for quantifying the enzyme dioxygenase activity relative to the kinetics of mineralization.

References

AGRITOX AFSSA-DIVE. (2007). Base de données sur les substances actives phytopharmaceutiques (Isoproturon). Site internet, http://www.dive.afssa.fr/agritox, dernier accès octobre 2007.

Alletto, L., Coquet, Y., Benoit, P., & Bergheaud, V. (2006). Effects of temperature and water content on degradation of isoproturon in three soil profiles. *Chemosphere, 64*(7), 1053–1061.

Alva, V. A., & Peyton, B. M. (2003). Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: Influence of pH and salinity. *Environmental Science and Technology, 37*(19), 4397–4402.

Amita, B., Anjali, S., Srivastava, A., Bali, R., Srivastava, P., & Govindra, S. (2005). Effect of temperature on adsorption-desorption of isoproturon on a clay soil. *Indian Journal of Weed Science, 37*(3&4), 247–250.

Badawi, N., Ronhede, S., Olsson, S., Kragelund, B. B., Johnsen, A. H., Jacobsen, O. S., et al. (2009). Metabolites of the phenylurea herbicides chlorotoluron, diuron, isoproturon and linuron produced by the soil fungus *Mortierella* sp. *Environmental Pollution, 157*(10), 2806–2812.

Beck, A. J., Harris, G. L., Howse, K. R., Johnston, A. E., & Jones, K. C. (1996). Spatial and temporal variation of isoproturon residues and associated sorption/desorption parameters at the field scale. *Chemosphere, 33*(7), 1283–1295.

Behera, B. C., & Bhunya, S. P. (1990). Genotoxic effect of isoproturon (herbicide) as revealed by three mammalian *in vivo* mutagenic bioassays. *Indian Journal of Experimental Biology, 28*(9), 862–867.

Bending, G. D., Lincoln, S. D., & Edmondson, R. N. (2006). Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. *Environmental Pollution, 139*(2), 279–287.

Bending, G. D., Lincoln, S. D., Sorensen, S. R., Morgan, J. A. W., Aamand, J., & Walker, A. (2003). In-field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative *Sphingomonas* spp. and soil pH. *Applied and Environment Microbiology, 69*(2), 827–834.

Bending, G. D., & Rodriguez-Cruz, M. S. (2007). Microbial aspects of the interaction between soil depth and biodegradation of the herbicide isoproturon. *Chemosphere, 66*(4), 664–671.

Bending, G. D., Shaw, E., & Walker, A. (2001). Spatial heterogeneity in the metabolism and dynamics of isoproturon degrading microbial communities in soil. *Biology and Fertility of Soils, 33*(6), 484–489.

Berger, B. M. (1999). Factors influencing transformation rates and formation of products of phenylurea herbicides in soil. *Journal of Agriculture and Food Chemistry, 47*(8), 3389–3396.

Bottcher, T., & Schroll, R. (2007). The fate of isoproturon in a freshwater microcosm with *Lemna minor* as a model organism. *Chemosphere, 66*(4), 684–689.

Caux, P. Y., Kent, R. A., Fan, G. T., & Grande, C. (1998). Canadian water quality guidelines for linuron. *Environmental Toxicology and Water Quality, 13*(1), 1–41.

Chhokar, R. S., & Malik, R. K. (2002). Isoproturon-resistant littleseed canarygrass (*Phalaris minor*) and its response to alternate herbicides. *Weed Technology, 16*(1), 116–123.

Coquet, Y., Ribiere, C., & Vachier, P. (2004). Pesticide adsorption in the vadose zone: A case study on Eocene and Quaternary materials in Northern France. *Pest Management Science, 60* (10), 992–1000.

Cox, L., Walker, A., & Welch, S. J. (1996). Evidence for the accelerated degradation of isoproturon in soils. *Pesticide Science, 48*(3), 253–260.

Cullington, J. E., & Walker, A. (1999). Rapid biodegradation of diuron and other phenylurea herbicides by a soil bacterium. *Soil Biology and Biochemistry, 31*(5), 677–686.

Dosnon-Olette, R., Couderchet, M., Oturan, M. A., Oturan, N., & Eullaffroy, P. (2011). Potential use of *Lemna Minor* for the phytoremediation of isoproturon and glyphosate. *International Journal of Phytoremediation, 13*(6), 601–612.

Dosnon-Olette, R., Trotel-Aziz, P., Couderchet, M., & Eullaffroy, P. (2010). Fungicides and herbicide removal in *Scenedesmus* cell suspensions. *Chemosphere, 79*(2), 117–123.

Dwivedi, S., Singh, B. R., Al-Khedhairy, A. A., & Musarrat, J. (2011). Biodegradation of isoproturon using a novel *Pseudomonas aeruginosa* strain JS-11 as a multi-functional bioinoculant of environmental significance. *Journal of Hazardous Materials, 185*(2–3), 938–944.

Eibisch, N., Schroll, R., & Fub, R. (2014). Effect of pyrochar and hydrochar amendments on the mineralization of the herbicide isoproturon in an agricultural soil. *Chemosphere*,. doi:10.1016/j.chemosphere.2014.11.074.

Eibisch, N., Schroll, R., Fub, R., Mikutta, R., Helfrich, M., & Flessa, H. (2015). Pyrochars and hydrochars differently alter the sorption of the herbicide isoproturon in an agricultural. *Chemosphere, 119*, 155–162.

El-Arfaoui, A., Boudesocque, S., Sayen, S., & Guillon, E. (2010). Terbumeton and isoproturon adsorption by soils: Influence of Ca^{2+} and K^{+} cations. *Journal of Pest Science, 2*, 131–133.

El-khattabi, K., Bouhaouss, A., Scrano, L., Lelario, F., & Bufo, S. A. (2007). Influence of humic fractions on retention of isoproturon residues in two Moroccan soils. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 42*(7), 851–856.

El-Sebai, T., Devers-Lamrani, M., Lagacherie, B., Rouard, N., Soulas, G., & Martin-Laurent, F. (2011). Isoproturon mineralization in an agricultural soil: Impact of temperature and moisture content. *Biology and Fertility of Soils, 47*(4), 427–435.

El-Sebai, T., Lagacherie, B., Cooper, J. F., Soulas, G., & Martin-Laurent, F. (2005). Enhanced isoproturon mineralisation in a clay silt loam agricultural soil. *Agronomy for Sustainable Development, 25*(2), 271–277.

El-Sebai, T., Lagacherie, B., Soulas, G., & Martin-Laurent, F. (2004). Isolation and characterisation of an isoproturon-mineralising *Methylopila* sp TES from French agricultural soil. *FEMS Microbiology Letters, 239*(1), 103–110.

El-Sebai, T., Lagacherie, B., Soulas, G., & Martin-Laurent, F. (2007). Spatial variability of isoproturon mineralizing activity within an agricultural field: Geostatistical analysis of simple physico-chemical and microbiological soil parameters. *Environmental Pollution, 145*(3), 680–690.

Environment-Agency. (2001). Pesticides 2000: A summary of monitoring of the aquatic environment in England and Wales. Environment Agency. www.environment-agency.gov.uk

Ertli, T., Marton, A., & Foldenyi, R. (2004). Effect of pH and the role of organic matter in the adsorption of isoproturon on soils. *Chemosphere, 57*(8), 771–779.

European Commission. (2001). Decision no. 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. European Commission.

Fenlon, K. A., Jones, K. C., & Semple, K. T. (2011). The effect of soil: Water ratios on the induction of isoproturon, cypermethrin and diazinon mineralisation. *Chemosphere, 82*(2), 163–168.

Fredrickson, J. K., Balkwill, D. L., Romine, M. F., & Shi, T. (1999). Ecology, physiology, and phylogeny of deep subsurface *Sphingomonas* sp. *Journal of Industrial Microbiology and Biotechnology, 23*(4–5), 273–283.

Gangwar, S. K., & Rafiquee, M. Z. A. (2007a). Kinetics of the acid hydrolysis of isoproturon in the absence and presence of sodium lauryl sulfate micelles. *Colloid and Polymer Science, 285* (5), 587–592.

Gangwar, S. K., & Rafiquee, M. Z. A. (2007b). Kinetics of the alkaline hydrolysis of isoproturon in CTAB and NaLS micelles. *International Journal of Chemical Kinetics, 39*(1), 39–45.

Gerecke, A. C., Scharer, M., Singer, H. P., Muller, S. R., Schwarzenbach, R. P., Sagesser, M., et al. (2002). Sources of pesticides in surface waters in Switzerland: pesticide load through waste water treatment plants-current situation and reduction potential. *Chemosphere, 48*(3), 307–315.

Greulich, K., Hoque, E., & Pflugmacher, S. (2002). Uptake, metabolism, and effects on detoxication enzymes of isoproturon in spawn and tadpoles of amphibians. *Ecotoxicology and Environmental Safety, 52*(3), 256–266.

Grundmann, S., Doerfler, U., Munch, J. C., Ruth, B., & Schroll, R. (2011). Impact of soil water regime on degradation and plant uptake behaviour of the herbicide isoproturon in different soil types. *Chemosphere, 82*(10), 1461–1467.

Gu, T., Zhou, C., Sorensen, S. R., Zhang, J., He, J., Yu, P., et al. (2013). The novel bacterial N-demethylase PdmAB is responsible for the initial step of N, N-dimethyl-substituted phenylurea herbicide degradation. *Applied and Environment Microbiology, 79*, 7846–7856.

Hangler, M., Jensen, B., Ronhede, S., & Sorensen, S. R. (2007). Inducible hydroxylation and demethylation of the herbicide isoproturon by *Cunninghamella elegans*. *FEMS Microbiology Letters, 268*(2), 254–260.

Hassan, N., & Nemat-Alla, M. (2005). Oxidative stress in herbicide-treated broad bean and maize plants. *Acta Physiologiae Plantarum, 27*(4), 429–438.

Hazarika, A., & Sarkar, S. N. (2001). Effect of isoproturon pretreatment on the biochemical toxicodynamics of anilofos in male rats. *Toxicology, 165*(2–3), 87–95.

Hoshiya, T., Hasegawa, R., Hakoi, K., Cui, L., Ogiso, T., Cabral, R., et al. (1993). Enhancement by non-mutagenic pesticides of GST-P positive hepatic foci development initiated with diethylnitrosamine in the rat. *Cancer Letters, 72*(1–2), 59–64.

Hussain, S., Arshad, M., Springael, D., Sorensen, S. R., Bending, G. D., Devers-Lamrani, M., et al. (2015). Abiotic and biotic processes governing the fate of phenylurea herbicides in soil: A review. *Critical Reviews in Environment Science and Technology, 45*, 1947–1998.

Hussain, S., Devers-Lamrani, M., El, Azhari N., & Martin-Laurent, F. (2011). Isolation and characterization of an isoproturon mineralizing *Sphingomonas* sp. strain SH from a French agricultural soil. *Biodegradation, 22*(3), 637–650.

Hussain, S., Devers-Lamrani, M., Spor, A., Rouard, N., Porcherot, M., Beguet, J., et al. (2013). Mapping field spatial distribution patterns of isoproturon-mineralizing activity over a three-year winter wheat/rape seed/barley rotation. *Chemosphere, 90*, 2499–2511.

Hussain, S., Sorensen, S. R., Devers-Lamrani, M., El-Sebai, T., & Martin-Laurent, F. (2009). Characterization of an isoproturon mineralizing bacterial culture enriched from a French agricultural soil. *Chemosphere, 77*(8), 1052–1059.

INERIS. (2007). Données technico-économiques sur les substances chimiques en France: ISOPROTURON. 27p. http://rsde.ineris.fr/

Issa, S., & Wood, M. (2005). Degradation of atrazine and isoproturon in surface and sub-surface soil materials undergoing different moisture and aeration conditions. *Pest Management Science, 61*(2), 126–132.

Johannesen, H., Sorensen, S. R., & Aamand, J. (2003). Mineralization of soil-aged isoproturon and isoproturon metabolites by *Sphingomonas* sp. strain SRS2. *Journal of Environmental Quality, 32*(4), 1250–1257.

Khadrani, A., Seigle-Murandi, F., Steiman, R., & Vroumsia, T. (1999). Degradation of three phenylurea herbicides (chlortoluron, isoproturon and diuron) by micromycetes isolated from soil. *Chemosphere, 38*(13), 3041–3050.

Knauert, S., Escher, B., Singer, H., Hollender, J., & Knauer, K. (2008). Mixture toxicity of three photosystem II inhibitors (atrazine, isoproturon, and diuron) toward photosynthesis of freshwater phytoplankton studied in outdoor mesocosms. *Environmental Science and Technology, 42*(17), 6424–6430.

Knauert, S., Singer, H., Hollender, J., & Knauer, K. (2010). Phytotoxicity of atrazine, isoproturon, and diuron to submersed macrophytes in outdoor mesocosms. *Environmental Pollution, 158* (1), 167–174.

Kristensen, K. E., Jacobsen, C. S., Hansen, L. H., Aamand, J., Morgan, J. A., Sternberg, C., et al. (2006). Genetic labelling and application of the isoproturon-mineralizing *Sphingomonas* sp. strain SRS2 in soil and rhizosphere. *Letters in Applied Microbiology, 43*(3), 280–286.

Kristensen, G. B., Sorensen, S. R., & Aamand, J. (2001). Mineralization of 2, 4-D, mecoprop, isoproturon and terbuthylazine in a chalk aquifer. *Pest Management Science, 57*(6), 531–536.

Kumar, S. (2010). Effect of 2, 4-D and isoproturon on chromosomal disturbances during mitotic division in root tip cells of *Triticum aestivum* L. *Cytology and Genetics, 44*(2), 79–87.

Larsen, L., Sorensen, S. R., & Aamand, J. (2000). Mecoprop, isoproturon, and atrazine in and above a sandy aquifer: Vertical distribution of mineralization potential. *Environmental Science and Technology, 34*(12), 2426–2430.

Liang, L., Lu, Y. L., & Yang, H. (2012). Toxicology of isoproturon to the food crop wheat as affected by salicylic acid. *Environmental Science and Pollution Research, 19*(6), 2044–2054.

Lopez-Munoz, M. J., Revilla, A., & Aguado, J. (2013). Heterogeneous photocatalytic degradation of isoproturon in aqueous solution: Experimental design and intermediate products analysis. *Catalysis Today, 209*, 99–107.

Lu, C. Y., Zhang, S., Miao, S. S., Jiang, C., Huang, M. T., Liu, Y., et al. (2015). Enhanced degradation of herbicide isoproturon in wheat rhizosphere by salicylic acid. *Journal of Agricultural and Food Chemistry, 63*(1), 92–103.

Maqbool, Z., Hussain, S., Imran, M., Mahmood, F., Shahzad, T., Ahmed, Z., et al. (2016). Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. *Environmental Science and Pollution Research*, http://dx.doi.org/10.1007/s11356-016-7003-8.

Mascolo, G., Lopez, A., James, H., & Fielding, M. (2001). By-products formation during degradation of isoproturon in aqueous solution. I: Ozonation. *Water Research, 35*(7), 1695–1704.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7* (9), 405–410.

Mosleh, Y. Y. (2009). Assessing the toxicity of herbicide isoproturon on *Aporrectodea caliginosa* (oligochaeta) and its fate in soil ecosystem. *Environmental Toxicology, 24*(4), 396–403.

Mosleh, Y. Y., Paris-Palacios, S., Couderchet, M., Biagianti-Risbourg, S., & Vernet, G. (2005). Effects of the herbicide isoproturon on metallothioneins, growth, and antioxidative defenses in the aquatic worm *Tubifex tubifex* (Oligochaeta, Tubificidae). *Ecotoxicology, 14*(5), 559–571.

Muller, K., Bach, M., Hartmann, H., Spiteller, M., & Frede, H. G. (2002). Point- and nonpoint-source pesticide contamination in the Zwester Ohm catchment, Germany. *Journal of Environmental Quality, 31*(1), 309–318.

Navarro, S., Bermejo, S., Vela, N., & Hernandez, J. (2009). Rate of loss of simazine, terbuthylazine, isoproturon, and methabenzthiazuron during soil solarization. *Journal of Agriculture and Food Chemistry, 57*(14), 6375–6382.

Nayak, A. S., Veeranagouda, Y., Lee, K., & Karegoudar, T. B. (2009). Metabolism of acenaphthylene via 1, 2-dihydroxynaphthalene and catechol by *Stenotrophomonas* sp RMSK. *Biodegradation, 20*(6), 837–843.

Nemat-Alla, M., & Hassan, N. (2007). Changes of antioxidants and GSH-associated enzymes in isoproturon-treated maize. *Acta Physiologiae Plantarum, 29*(3), 247–258.

Nitschke, L., Wilk, A., Schussler, W., Metzner, G., & Lind, G. (1999). Biodegradation in laboratory activated sludge plants and aquatic toxicity of herbicides. *Chemosphere, 39*(13), 2313–2323.

Orton, F., Lutz, I., Kloas, W., & Routledge, E. J. (2009). Endocrine disrupting effects of herbicides and: In vitro and in vivo evidence. *Environmental Science and Technology, 43*(6), 2144–2150.

Paris-Palacios, S., Mosleh, Y. Y., Almohamad, M., Delahaut, L., Conrad, A., Arnoult, F., et al. (2010). Toxic effects and bioaccumulation of the herbicide isoproturon in Tubifex tubifex (Oligocheate, Tubificidae): A study of significance of autotomy and its utility as a biomarker. *Aquatic Toxicology, 98*(1), 8–14.

Parra, S., Sarria, V., Malato, S., Péringer, P., & Pulgarin, C. (2000). Photochemical versus coupled photochemical-biological flow system for the treatment of two biorecalcitrant herbicides: metobromuron and isoproturon. *Applied Catalysis B: Environmental, 27*(3), 153–168.

Parris, G. E. (1980). Environmental and metabolic transformations of primary aromatic amines and related compounds. *Residue Reviews, 76*, 1–30.

Pérés, F., Florin, D., Grollier, T., FeurtetMazel, A., Coste, M., Ribeyre, F., et al. (1996). Effects of the phenylurea herbicide isoproturon on periphytic diatom communities in freshwater indoor microcosms. *Environmental Pollution, 94*(2), 141–152.

Perrin-Ganier, C., Schiavon, F., Morel, J. L., & Schiavon, M. (2001). Effect of sludge-amendment or nutrient addition on the biodegradation of the herbicide isoproturon in soil. *Chemosphere, 44*(4), 887–892.

Perrin-Ganier, C., Schiavon, M., Portal, J. M., & Babut, M. (1995). Dégradation de l'isoproturon et disponibilité de ses résidus dans le sol. *Weed Research, 35*(4), 257–263.

Pieuchot, M., Perrin Ganier, C., Portal, J. M., & Schiavon, M. (1996). Study on the mineralization and degradation of isoproturon in three soils. *Chemosphere, 33*(3), 467–478.

Racke, K. D., & Coats, J. R. E. (1990). *Enhanced biodegradation of pesticides in the environment*. Washington, DC: American Chemical Society.

Reddy, P. A. K., Reddy, P. V. L., Sharma, V. M., Kumari, V. D., & Subrahmanyam, M. (2010). Photocatalytic degradation of isoproturon pesticide on C, N and S doped TiO_2. *Journal of Water Resource and Protection, 2*(3), 235–244.

Reddy, P. A. K., Srinivas, B., Durgakumari, V., & Subrahmanyam, M. (2012). Solar photocatalytic degradation of the herbicide isoproturon on a Bi-TiO_2/zeolite photocatalyst. *Toxicological and Environmental Chemistry, 94*, 512–524.

Reddy, P. A. K., Srinivas, B., Kala, P., Kumari, V. D., & Subrahmanyam, M. (2011). Preparation and characterization of Bi-doped TiO_2 and its solar photocatalytic activity for the degradation of isoproturon herbicide. *Materials Research Bulletin, 46*, 1766–1771.

Reid, B. J., Papanikolaou, N. D., & Wilcox, R. K. (2005). Intrinsic and induced isoproturon catabolic activity in dissimilar soils and soils under dissimilar land use. *Environmental Pollution, 133*(3), 447–454.

Reid, B. J., Pickering, F. L., Freddo, A., Whelan, M. J., & Coulon, F. (2013). Influence of biochar on isoproturon partitioning and bioaccessibility in soil. *Environmental Pollution, 181*, 44–50.

Rigaud, J. P., Lebreton, J. C. (2004). Points de repère - Blé: désherbage de post-levée. Chambre d'Agriculture de Mayenne. http://www.mayenne.chambagri.fr/services/documentation/ble_desherbage_post_levee.pdf, denier accès octobre 2007.

Roberts, S. J., Walker, A., Cox, L., & Welch, S. J. (1998). Isolation of isoproturon-degrading bacteria from treated soil via three different routes. *Journal of Applied Microbiology, 85*(2), 309–316.

Rodriguez-Cruz, M. S., Jones, J. E., & Bending, G. D. (2006). Field-scale study of the variability in pesticide biodegradation with soil depth and its relationship with soil characteristics. *Soil Biology and Biochemistry, 38*(9), 2910–2918.

Ronhede, S., Jensen, B., Rosendahl, S., Kragelund, B. B., Juhler, R. K., & Aamand, J. (2005). Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. *Applied and Environment Microbiology, 71*(12), 7927–7932.

Ronhede, S., Sorensen, S. R., Jensen, B., & Aamand, J. (2007). Mineralization of hydroxylated isoproturon metabolites produced by fung. *Soil Biology and Biochemistry, 39*(7), 1751–1758. doi:10.1016/j.soilbio.2007.01.037.

Rubio, M. I. M., Gernjak, W., Alberola, O., Galvez, J. B., Fernandez-Ibanez, P., & Rodriguez, S. (2006). Photo-Fenton degradation of alachlor, atrazine, chlorfenvinphos, diuron, isoproturon and pentachlorophenol at solar pilot plant. *International Journal of Environment and Pollution, 27*(1–3), 135–146.

Scheunert, I., & Reuter, S. (2000). Formation and release of residues of the C-14-labelled herbicide isoproturon and its metabolites bound in model polymers and in soil. *Environmental Pollution, 108*(1), 61–68.

Schmitt-Jansen, M., & Altenburger, R. (2005). Toxic effects of isoproturon on periphyton communities - a microcosm study. *Estuarine, Coastal and Shelf Science, 62*(3), 539–545.

Schroll, R., Becher, H. H., Dorfler, U., Gayler, S., Hartmann, H. P., & Ruoss, J. (2006). Quantifying the effect of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils. *Environmental Science and Technology, 40*(10), 3305–3312.

Sharma, M. V. P., Durgakumari, V., & Subrahmanyam, M. (2008a). Solar photocatalytic degradation of isoproturon over TiO_2/H-MOR composite systems. *Journal of Hazardous Materials, 160*(2–3), 568–575.

Sharma, M. V. P., Kumari, V. D., & Subrahmanyam, A. (2008b). Photocatalytic degradation of isoproturon herbicide over TiO_2/Al-MCM-41 composite systems using solar light. *Chemosphere, 72*(4), 644–651.

Sharma, M. V. P., Kumari, V. D., & Subrahmanyam, M. (2010). TiO_2 supported over porous silica photocatalysts for pesticide degradation using solar light: Part 2. Silica prepared using acrylic acid emulsion. *Journal of Hazardous Materials, 175*(1–3), 1101–1105.

Sharma, M. V. P., Lalitha, K., Durgakumari, V., & Subrahmanyam, M. (2008c). Solar photocatalytic mineralization of. isoproturon over TiO_2/HY composite systems. Solar Energy Mater. *Solar Cells, 92*(8), 992.

Sharma, P. M. V., Sadanandam, G., Ratnamala, A., Durga Kumari, V., & Subrahmanyam, M. (2009). An efficient and novel porous nanosilica supported TiO_2 photocatalyst for pesticide degradation using solar light. *Journal of Hazardous Materials, 171*(1–3), 626–633.

Shi, S. J., & Bending, G. D. (2007). Changes to the structure of Sphingomonas spp. communities associated with biodegradation of the herbicide isoproturon in soil. *FEMS Microbiology Letters, 269*(1), 110–116.

Si, Y., Wang, M., Tian, C., Zhou, J., & Zhou, D. (2011). Effect of charcoal amendment on adsorption, leaching and degradation of isoproturon in soils. *Journal of Contaminant Hydrology, 123*(1–2), 75–81.

Si, Y. B., Zhang, J., Wang, S. Q., Zhang, L. G., & Zhou, D. M. (2006). Influence of organic amendment on the adsorption and leaching of ethametsulfuron-methyl in acidic soils in China. *Geoderma, 130*(1–2), 66–76.

Sorensen, S. R., & Aamand, J. (2003). Rapid mineralisation of the herbicide isoproturon in soil from a previously treated Danish agricultural field. *Pest Management Science, 59*(10), 1118–1124.

Sorensen, S. R., Bending, G. D., Jacobsen, C. S., Walker, A., & Aamand, J. (2003). Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. *FEMS Microbiology Ecology, 45*(1), 1–11.

Sorensen, S. R., Ronen, Z., & Aamand, J. (2001). Isolation from agricultural soil and characterization of a Sphingomonas sp able to mineralize the phenylurea herbicide isoproturon. *Applied and Environment Microbiology, 67*(12), 5403–5409.

Sorensen, S. R., Ronen, Z., & Aamand, J. (2002). Growth in coculture stimulates metabolism of the phenylurea herbicide isoproturon by Sphingomonas sp strain SRS2. *Applied and Environment Microbiology, 68*(7), 3478–3485.

Soulas, G. (1993). Evidence for the existence of different physiological groups in the microbial community responsible for 2, 4-D mineralization in soil. *Soil Biology and Biochemistry, 25*(4), 443–449.

Steiman R, Seigle-Murandi F, Benoit-Guyod JL, Merlin G, Khadri M (1994) Assessment of isoproturon degradation by fungi. In Proceedings of 5th International Workshop, Brussels (pp. 229–235).

Sun, J. Q., Huang, X., Chen, Q. L., Liang, B., Qiu, J. G., Ali, S. W., et al. (2009). Isolation and characterization of three *Sphingobium* sp. strains capable of degrading isoproturon and cloning of the catechol 1, 2-dioxygenase gene from these strains. *World Journal of Microbiology and Biotechnology, 25*(2), 259–268.

Tian, C., M-d, Wang, & Y-b, Si. (2010). Influences of charcoal amendment on adsorption-desorption of isoproturon in soils. *Agricultural Sciences in China, 9*(2), 257–265.

Tixier, C., Sancelme, M., Ait-Aissa, S., Widehem, P., Bonnemoy, F., Cuer, A., et al. (2002). Biotransformation of phenylurea herbicides by a soil bacterial strain, *Arthrobacter* sp. N2: structure, ecotoxicity and fate of diuron metabolite with soil fungi. *Chemosphere, 46*(4), 519–526.

Turnbull, G. A., Ousley, M., Walker, A., Shaw, E., & Morgan, J. A. W. (2001). Degradation of substituted phenylurea herbicides by *Arthrobacter globiformis* strain D47 and characterization of a plasmid-associated hydrolase gene, puhA. *Applied and Environment Microbiology, 67*(5), 2270–2275.

Vallotton, N., Eggen, R. I. L., & Chevre, N. (2009). Effect of sequential isoproturon pulse exposure on *Scenedesmus vacuolatus*. *Archives of Environmental Contamination and Toxicology, 56*(3), 442–449.

Vieublé-Gonod, L., Benoit, P., Cohen, N., & Houot, S. (2009). Spatial and temporal heterogeneity of soil microorganisms and isoproturon degrading activity in a tilled soil amended with urban waste composts. *Soil Biology and Biochemistry, 41*(12), 2558–2567.

Vrielynck, L., Dupuy, N., Kister, J., & Nowogrocki, G. (2006). Molecular structure and vibrational spectroscopy of isoproturon. *Journal of Molecular Structure, 788*(1–3), 232–237.

Vroumsia, T., Steiman, R., SeigleMurandi, F., BenoitGuyod, J. L., & Khadrani, A. (1996). Biodegradation of three substituted phenylurea herbicides (chlorotoluron, diuron, and isoproturon) by soil fungi. A comparative study. *Chemosphere, 33*(10), 2045–2056.

Walker, A., Bromilow, R. H., Nicholls, P. H., Evans, A. A., & Smith, V. J. R. (2002). Spatial variability in the degradation rates of isoproturon and chlorotoluron in a clay soil. *Weed Research, 42*(1), 39–44.

Walker, A., & Welch, S. J. (1991). Enhanced degradation of some soil-applied herbicides. *Weed Research, 31*(1), 49–57.

White, D. C., Sutton, S. D., & Ringelberg, D. B. (1996). The genus Sphingomonas: Physiology and ecology. *Current Opinion in Biotechnology, 7*(3), 301–306.

Widenfalk, A., Bertilsson, S., Sundh, I., & Goedkoop, W. (2008). Effects of pesticides on community composition and activity of sediment microbes—responses at various levels of microbial community organization. *Environmental Pollution, 152*(3), 576–584.

Widenfalk, A., Svensson, J. M., & Goedkoop, W. (2004). Effects of the pesticides captan, deltamethrin, isoproturon, and pirimicarb on the microbial community of a freshwater sediment. *Environmental Toxicology and Chemistry, 23*(8), 1920–1927.

Yin, X. L., Jiang, L., Song, N. H., & Yang, H. (2008). Toxic reactivity of wheat (*Triticum aestivum*) plants to herbicide isoproturon. *Journal of Agriculture and Food Chemistry, 56*(12), 4825–4831.

Yu, P., Tan, Z. C., Meng, S. H., Lu, S. W., Lan, X. Z., Sun, L. X., et al. (2003). Low-temperature heat capacities and thermodynamic properties of crystalline isoproturon. *Journal of Thermal Analysis and Calorimetry, 74*(3), 867–874.

Zhang, J., Hong, Q., Li, Q., Li, C., Cao, L., Sun, J. Q., et al. (2012). Characterization of isoproturon biodegradation pathway in *Sphingobium* sp. YBL2. *International Biodeterioration and Biodegradation, 70*, 8–13.

Endosulfan a Cyclodiene Organochlorine Pesticide: Possible Pathways of Its Biodegradation

Dileep K. Singh and N. Sarat Singh

1 Introduction

With rapidly increasing world population and shrinking of cultivable areas, enhancing the food production in order to meet the growing demand has become a major challenge with the modern agriculture. Hence, in order to meet the increasing demand for food supply, various new methods and techniques have been employed from time to time. The use of agrochemicals is one of them. Agrochemicals refer to the group of chemicals used in the agriculture, either natural or synthetics, such as pesticides, fertilizers, hormones and other growth agents in order to boost up crop production.

Pesticides play an important role in ensuring food security for the growing world population. Even today, with so much advancement in agricultural sciences, loss due to pest and diseases ranges from 10 to 90 % with an average of 35–40 % for all potential food and fibre crops (Peshin 2002). Therefore, programs have been developed to reduce the dependency of production on synthetic pesticides just to minimise its environmental effect, and to maintain the efficacy of crop protection products in order to maintain sustainable crop production at higher intensities (Oerke and Dehne 2004). In fact, use of genetically engineered crops in last decade has considerably decrease the pesticide use in both developed and developing countries (Qaim and Zilberman 2003). During the first 50 years of the introduction of the synthetic organic compound, the use of pesticide in agriculture has increased so dramatically that now it amounts to some 2.56 billion kg per year. Highest growth rate of 12 % per year occur in pesticide market in 1960s (Daly et al. 1998).

Pesticides in general can be categorized as insecticides, fungicides and herbicides. Insecticides are further classified as organophosphorus, organochlorine,

D.K. Singh (✉) · N. Sarat Singh
Department of Zoology, University of Delhi, Delhi 110007, India
e-mail: dileepksingh@gmail.com

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_5

carbamates, pyrethroids etc. Besides, there are also minor pesticides, such as nematicides, acaricides, rodenticides, mollucicides and larvicides which are used by the farmers to kill the pests for protection of crop yields and the domestic animals.

2 Organochlorine Pesticide

Organochlorine pesticides are synthetic organic chemicals containing at least one covalently bonded chlorine atom. They were first introduced in the 1940s and 1950s as insecticides, such as DDT, methoxychlor, chlordane, heptachlor, aldrin, dieldrin, endrin, toxaphene, mirex, and lindane. The chlorinated hydrocarbons are neurotoxicants and cause acute damage to insects by interfering with the transmission of nerve impulses. The presence of chlorine atoms makes them denser than water and highly insoluble, hence chlorinated pesticides persist in the environment for a long time.

DDT, as well as other organochlorines, was used extensively from the 1940s through the 1960s in agriculture and mosquito control, particularly in the World Health Organization (WHO) malaria control programs. The cyclodiene insecticides, such as chlordane were used extensively as termiticides into the 1980s, but later removed from the market due to measurable residue levels penetrating into interiors and allegedly causing health problems. Its persistence, initially considered a desirable attribute, later on became the basis for public concern. The publication of Rachel Carson's book *The Silent Spring* in 1962, heightened health concern which eventually led to the ban of DDT and other chlorinated insecticides in the United States in 1972 (Hodgson 2004). However, some chlorinated compounds, like endosulfan, are still widely used throughout the world.

3 Endosulfan

Endosulfan (6,7,8,9,10,10-hexa chloro-1,5,5a,6,9,9a-hexahydro—6,9-methano-2, 4,3-benzodioxathiepine-3-oxide) is a cyclodiene organochlorine pesticide. Which is used as a broad spectrum insecticide against many insects and mites in agriculture and allied sectors. It is used in vegetables, fruits, paddy, cotton, cashew, tea, coffee, and tobacco and timber crops from insects. Besides, it is also used as wood preservatives and to control tsetse flies and termites.

Worldwide use of endosulfan was increased with the ban/restriction in use of the more persistent organochlorine pesticides like DDT and Endrin. It is reported to be one of the most commonly used pesticides in India in recent years, particularly on rice and cotton against thrips, stem borer, whorl maggot, case worm, boll worm and bud worm (Jayashree and Vasudevan 2007).

3.1 Endosulfan Production

Endosulfan is produced by the Diels-Alder reaction of hexachlorocyclopentadiene with *cis*-butene-1, 4-diol and subsequent reaction of adduct with thionyl chloride in the presence of an inert solvent like carbon tetrachloride. Technical endosulfan is a mixture of two stereoisomers, designated as α- and β-endosulfan in ratio of 7:3. They are conformational isomers arising from the pyramidal stereochemistry of sulphur atom. The α-isomer is thermodynamically more stable than its β-isomer which gets irreversibly converted into α-form, although the conversion is slow.

Endosulfan can enter the air as spray droplets and can be carried away from the target site during application. This can affect the non-target organisms and also pollute the surrounding environment. Endosulfan was first noticed in the Artic air in 1986–87, since then its concentration was found to be increasing every year. The concentration measured in 1993–97 being 0.0042–0.0047 ng/m^3. Besides, it has been described as one of the most abundant and ubiquitous air contaminant in North America (GFEA-U 2007).

India is the world's largest producer and user of endosulfan (Li and Macdonald 2005). Three major producer companies and Excel Crop Care, Hindustan Insecticide Limited, and Coromandel Fertilizers, produce 4500 tonnes annually for domestic use and another 4000 tonnes for export (Venkatraman 2004). Germany is the second largest producer of endosulfan by Bayer Crop Science, producing about 4000 tonnes annually (GFEA-U 2007), followed by China (Li et al. 2007).

3.2 Physical and Chemical Properties of Endosulfan

In pure form, endosulfan exists as colourless crystals, but the technical product is brownish crystals with slight odour of sulphur dioxide. Technically, endosulfan is a mixture of two isomers- α-endosulfan and β-endosulfan in the ratio 7:3. Technical grade endosulfan contains 94 % α-endosulfan and β-endosulfan along with other related compounds, like endosulfan alcohol, endosulfan ether and endosulfan sulphate.

Melting point: α-endosulfan: 109.2 °C, β-endosulfan: 213.3 °C, Technical endosulfan (mixture of isomers): 70–100 °C

Relative density: 1.745 g/cm^3

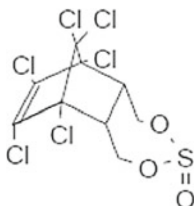
Vapour pressure: Endosulfan (mixture α-β endosulfan): 1.7×10^{-3} Pa, α -endosulfan: 1.9×10^{-3} Pa, β -endosulfan: 9.2×10^{-5} Pa

Volatility: α-endosulfan: 1.48 Pa × m^3 × mol^{-1} at 24 °C, β-endosulfan: 0.07 Pa × m^3 × mol^{-1} at 24 °C

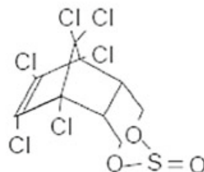
Appearance, Physical state and colour: Pure: white crystalline solid, Technical: yellow crystalline solid.

Odour: Pure: none, Technical: slight specific odour.
Chemical structure:

α-endosulfan



β-endosulfan



Solubility in water: α-endosulfan: 0.41 mg L^{-1}, β-endosulfan: 0.23 mg L^{-1}, Endosulfan (mixture of isomers): 0.63 mg L^{-1}

Solubility in organic solvents g 100 ml^{-1} at 20 °C: Acetonitrile—51.3, Acetone—116.4, Carbon tetrachloride —42.8, Dichloromethane—200.7, Ethyl acetate—100.9, n-Heptane—20.6, Methanol—16.8, n-Octanol—13.1, Toluene—226, p-Xylene—104.4.

Partition coefficient n-octanol/water: n-Octanol/Water partition coefficient = 47,200. Log P_{ow} = 4.67 (High partition coefficient risk for bioaccumulation must be contemplated for Endosulfan)

Stability in water—Hydrolysis rate in water

α-Endosulfan

pH	Temperature (°C)	DT_{50} (days)
5	25	>200
7	25	19
9	25	0.26

β-Endosulfan

pH	Temperature (°C)	DT_{50} (days)
5	25	>200
7	25	10.7
9	25	0.17

Stability in air, photochemical degradation, identity of breakdown product (s): Irradiation experiments in the gas phase have clearly shown that photodegradation processes of α- and β-endosulfan and endosulfan-sulphate can readily occur under natural conditions. The substances were converted to photoproducts and simple inorganic compounds (e.g. CO_2, CO or HCl).

Substance	t ½ in h
α-endosulfan	6.4
β-endosulfan	2.7
Endosulfan-sulphate	3.7

Endosulfan sulphate is the main photochemical degradation product in air, probably due to photo-oxidation. The half-life values are not relevant for outdoor conditions, as the products were exposed to intensive radiation. Hence, they cannot be taken as estimates of the persistence of endosulfan or endosulfan sulphate in environmental conditions which could be much longer.

3.3 Toxicity of Endosulfan

3.3.1 Toxicity Classification

The EPA, USA classifies endosulfan as Category 1b: highly hazardous, based on the LD_{50} value of 30 mg kg^{-1} for female rat, while World Health Organization (WHO) classifies it as Class II: moderately hazardous based on the LD_{50} of 80 mg kg^{-1} for rat. The Industrial Toxicological Research Centre (ITRC), India has classified it as extremely hazardous compound.

3.3.2 Mode of Action

Endosulfan has a strong affinity with the GABA (γ-aminobutyric acid) receptors in the brain and act as non-competitive GABA inhibitors. Binding of GABA to its receptors induces the uptake of chloride ions by the neurons and blockage of the uptake by endosulfan may result in a state of uncontrolled excitation (UNEP/FAO 2007). The LD_{50} of endosulfan varies widely depending on the route of administration, species, vehicle and sex of the animal. Endosulfan is generally highly toxic after oral and inhalation exposure (McGregor 1998).

The toxicity of endosulfan can be broadly discussed under two headings:

3.3.3 Acute Toxicity

Endosulfan and its isomers show high acute toxicity after oral administration, followed by dermal exposure and inhalation. The clinical signs of poisoning include piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching, and convulsions (JMPR 2008). Cows, which grazed in a sprayed field, may cause reversible blindness. The animals completely recovered only after a

month following the exposure. In an accidental exposure, sheep and pigs, grazing on a sprayed field, suffered from a lack of muscle co-ordination and blindness (Smith 1991).

LD_{50}s for endosulfan are:

- Oral LD50 rat = 10–23 mg kg^{-1} (female); 48–160 mg kg^{-1} (male)
- Dermal LD50 rat = 500 mg kg^{-1} (female); > 4000 mg kg^{-1} (male)
- Inhalation LC50 rat = 0.0126 mg l^{-1} (female); 0.0345 mg l^{-1} (male) (GFEA-U 2007).

It was found that α-isomers of endosulfan are more toxic than its β-counterparts. Toxicity of endosulfan may also be influenced by species, gender and by level of protein in the diet as well. Rats, which have been deprived of protein in their diets, are susceptible twice to the toxic effects of endosulfan as compared to the rats with normal balance diet (ATSDR 2000). Solvents and/or emulsifiers, used with endosulfan in formulated products, also influence its absorption into the system via all routes. Technical endosulfan is usually absorbed very slowly and incompletely into the body, whereas absorption is more rapid in the presence of alcohols, oils and emulsifiers.

3.3.4 Chronic Toxicity

The chronic NOAEL and LOAEL of endosulfan are:

- NOAEL = 0.57 mg kg^{-1} day^{-1} (female dog), 0.67 mg kg^{-1} day^{-1} (male dog)
- LOAEL = 1.75 mg kg^{-1} (dog)

Teratogenic Effects

An oral dose of 2.5 mg kg^{-1} day^{-1} resulted in the normal reproduction in rats in a three-generational study, but higher doses (5 and 10 mg kg^{-1} day^{-1}) resulted in abnormalities in bone development in the off springs (U.S. Agency for Toxic Substances and Disease Registry 1990; Smith 1991). However, teratogenicity effects in humans are unlikely at expected exposure levels.

Mutagenic Effects

Endosulfan is highly mutagenic to bacterial and yeast cells (U.S. Agency for Toxic Substances and Disease Registry 1990). The metabolites of endosulfan have also shown the ability to cause cellular changes (Smith 1991). Exposure to endosulfan may cause DNA damage and mutation in humans. A 2007 study by the California Department of Public Health found that women, who lived near farm fields sprayed

with endosulfan during the first eight weeks of pregnancy are several times more likely to give birth to children with autism. These results are highly preliminary as small number of women's and children were examined (Pesticide Action Network 2008).

Endosulfan is also found to damage human red blood cells (RBC) at concentrations of 1 ppb–1 ppm (Daniel et al. 1986). Endosulfan is hepatotoxic (Anonymous 1997), mutagenic, clastogenic to human and can affect the cell-cycle kinetics (Quijano 2000). Endosulfan has been shown to cause chromosomal aberrations in hamster and mouse and sex-linked mutations in Drosophila (Sang and Petrovic 1999; Chowdhari et al. 2001). It is also known to cause mutations in other mammals too.

Genotoxic Effect

Both α-endosulfan and β-endosulfan are genotoxic to HepG2 cells (Lu et al. 2000). Several studies also show that endosulfan is highly genotoxic (Pandey et al. 1990, 2006; Neuparth et al. 2006). Studies in human cells both in vitro and in- vivo also showed that endosulfan caused the occurrence of sister chromatid exchanges, indicating chromosomal damage (Sobti 1983). Very recently, a team of researchers in Japan found further evidence of endosulfan genotoxicity with respect to sister chromatid exchanges, micronuclei and DNA strand breaks as detected by single cell gel electrophoresis as biomarkers (Yuquan 2000).

Carcinogenic Effects

No accurate data related to the carcinogenicity of endosulfan in human are available, but from field level reports, endosulfan is highly suspected for carcinogenic properties in human beings, especially in cases of chronic exposure (Quijano 2000). In mice and rats, the males of both groups had high mortality rate, but no conclusions could be drawn about its carcinogenicity (Anonymous 2002). However, the females of both species failed to develop any carcinogenic symptoms even after 78 weeks of feeding with diets containing about 23 mg kg^{-1} day^{-1} of endosulfan. The highest tolerated dose of endosulfan did not cause any increased incidence of tumors in mice over 18 months, and a later study also showed no evidence of carcinogenic activity in mice or rats (Smith 1991; Anonymous 2002).

Therefore, endosulfan is not listed as known, probable, or possible carcinogen by the EPA, IARC, or other agencies. However, in vitro assays have shown that endosulfan induces proliferation of human breast estrogen sensitive MCF7 cells (Soto et al. 1994; Preziosi 1998), which increases the chances of breast cancer. In Kerala, India, endosulfan has been linked to hundreds of deaths and disorders among cashew nut plantation workers and villagers in Kasaragod province, where aerial spraying of endosulfan was carried out for over two decades (Thanal

Conservation Action and Information Network 2001). Alarmingly high levels of endosulfan residues have been detected in the blood and breast milk of villagers. Cancers and disorders of the reproductive and central nervous systems are very common in the area. A survey conducted for 123 houses found 49 cancer cases, 43 psychiatric, 23 epileptics, 9 congenital abnormalities and 23 mental retardation cases (Joshi 2001).

Organ Toxicity

The organs, most likely to be affected by endosulfan exposure, include kidneys, liver, blood, and the parathyroid gland. Thyroid follicular damage in mouse has been reported by Sang and Petrovic (1999). Besides, endosulfan is known to damage the endocrine system, nervous system, circulatory, reproductory, respiratory and excretory systems and developing foetus (Naqvi and Vaishnavi 1993; Paul et al. 1995; Sinha et al. 1995; Reuber 1981).

Reproductive Effects

Rats, fed with 2.5 mg kg^{-1} day^{-1} of endosulfan daily for three generations, showed no observable reproductive effects, but 5.0 mg kg^{-1} day^{-1} increased the mortality and resorption (Smith 1991). Female mice, fed with the endosulfan for 78 weeks at 0.1 mg kg^{-1} day^{-1}, showed damage in their reproductive organs (Anonymous 2002). Oral dosage for 15 days at 10 mg kg^{-1} day^{-1} in male rats caused damage to the seminiferous tubules and also lowered testes weights (Hurt 1991). Endosulfan exposure in male children may delay sexual maturity indicating interference with sex-hormone synthesis (Saiyed et al. 2003).

Endocrine Disruptive Action

An in vitro study shows that endosulfan is estrogenic (in the E-SCREEN assay). α -endosulfan competes with [3H] 17b—estradiol for binding with the estrogen receptor. Endosulfan sulfate inhibits binding of [3H] R5020 to the progesterone receptor by 40–50 %. Low levels of endosulfan (1 nM, 0.41 ppb) can incompletely inhibit the human sperm acrosome reaction, initiated by progesterone and glycine. β-endosulfan and endosulfan sulfate decreased b-galactosidase activity of progesterone (Jin et al. 1997).

3.3.5 Toxicity of Metabolites

Like endosulfan, its metabolites were more or less toxic according to the vehicle used and the species exposed. In general, the toxicity of the lactone and sulfate

metabolites was similar to or less than that of the parent compound, while the hydro ether, ether, and, in particular, the diol were far less toxic. The clinical signs of poisoning were similar to those induced by the parent compound which included piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching, and convulsions (McGregor 1998).

The major metabolite, endosulfan sulfate, was found to be very toxic to aquatic organisms like golden ide (*Leuciscusidus melanotus*), goldfish (*Carassius auratus*), and for guppy (*Poecilia reticulata*) $10\ \mu g\ L^{-1} < LC_{50} < 100\ \mu g\ L^{-1}$ were reported in literature. Endosulfan sulfate affects green algae (*Chlorella vulgaris*) at concentrations in the range of 0.5–10 $\mu g\ L^{-1}$. It also interferes with reproduction in *Daphnia*, causing a significant decrease in number of off springs, with reduced size of females and an increased proportion of males. It causes a decrease in moulting frequency, early development arrest, and a range of embryo deformities, including underdeveloped second antennae, curved, incurved or non-existent shell spine and other morphological alterations of the carapace. The concentrations of endosulfan sulphate, that affects reproductive outcome, are significantly lower than for similar effects caused by endosulfan isomers ($9.20 \times 10\ \mu g\ L^{-1}$ compared to $120 \times 10\ \mu g\ L^{-1}$) (Palma et al. 2009). The reported oral LD50 of endosulfan sulphate in rat is 18 mg kg^{-1} body weight.

3.4 *Ecological Effect of Endosulfan*

Endosulfan is very toxic to almost all kinds of organisms. The European Union risk statement of the compound includes: very toxic, dangerous for the environment, harmful in contact with skin, very toxic by inhalation, very toxic if swallowed and, very toxic to aquatic organisms may cause long term effect in the aquatic environment.

3.4.1 Effect of Endosulfan in the Aquatic Ecosystem

Endosulfan can enter the aquatic ecosystem either through runoff desire heavy rainfall after the application, accidental introduction during washing of endosulfan containers or instrument of application or intentionally used for fishing purpose. It is extremely toxic to fish (Goebel et al. 1982) and its continuous use can lead to disruption in the aquatic food chain (Dutta and Arends 2003).

In fish, it causes marked changes in sodium and potassium concentration, decreases blood calcium and magnesium levels, and inhibits brain ATPase and acetylcholinesterase. Hence, accidental or intentional exposure of endosulfan can cause mass killing of fishes (Naqvi and Vaishnavi 1993; Dutta and Arends 2003). Moreover, genotoxic effect of endosulfan on fishes has been also reported by Sharma et al. (2007).

In aquatic invertebrates, endosulfan causes decease in adenylate energy charge, oxygen consumption, haemolymph amino acids, succinate dehydrogenase, heartbeat (in muscle) and altered osmoregulation (Naqvi and Vaisnavi 1993). It also causes impairment of feeding, growth rates, embryonic development and reproduction rate (GFEA-U 2007). Therefore, the use of endosulfan in the paddy field and near the water bodies may cause significant imbalance in the aquatic ecosystem.

In amphibians, sub-lethal exposure of endosulfan significantly increases the vulnerability of tadpoles to the predators (Broomhall 2002). It also reduces their size and feeding behavior (Broomhall and Shine 2003). The low concentration of endosulfan interferes with the pheromone system of both male and female newts, disrupting mate selection and causing reduced mating success (Park et al. 2001; Park and Propper 2002). Besides, endosulfan has been also reported to affect the reptiles, snails and even the aquatic plants like phytoplankton, algae etc. population when exposed at minimum concentration level.

3.4.2 Effect of Endosulfan in the Terrestrial Ecosystem

Endosulfan is highly toxic to birds and act as immunosuppressive agent (Bhattacharya et al. 1993; Kurkure et al. 1993; Garg et al. 2004). Exposure of chicken egg at extremely low concentration adversely affects liver and brain enzymes, decreases DNA and RNA in brain and suppresses the immune system (Pushpanjali et al. 2005). It is highly toxic to bees and affects the olfactory learning even at sublethal level (Quijano 2002; NIOH 2003).

Endosulfan causes 60.5 % decrease in population of actinomycetes in just 10 days after treatment in soil (Vig et al. 2008). It also affects the population of many important soil invertebrates, arthropods, earthworms and many beneficial soil fungi. Even the permeability of the root membrane is greatly reduced due to endosulfan exposure, resulting in coiling of root radicals, inhibition of root growth, stunting of shoots and burning of the margin and tips of the leaves (IPCS 1984).

The effect of endosulfan on non-target species can be swift and devastating. Farmers in Benin have observed birds and frogs dying following consumption of insects sprayed with endosulfan. Fields used to smell awful two or three days after spraying of endosulphan, because, living organisms are killed and start to rot (Ton et al. 2000).

3.4.3 Bioaccumulation

Bioaccumulation is a general term for the accumulation of substances in an organism through respiration, food intake, absorption through the skin, etc. which results higher accumulation of the substance in the organism than its concentration in the surrounding environment. The Stockholm and US Conventions recognise a chemical as bioaccumulative, if it has a bioaccumulation or bioconcentration factor

(BCF) > 5000 in an aquatic species, or a log $K_{ow} > 5$. However, EU recognizes bioaccumulation, when the BCF is >2000 (Kelly et al. 2007).

Estimates of log K_{ow} of endosulfan isomers and its metabolite endosulfan sulphate are: α-endosulfan = 4.65, β-endosulfan = 4.34 and endosulfan sulphate = 3.77 (GFEA-U 2007).

In aquatic ecosystems, endosulfan residues tend to reach a plateau level in tissues. Schoettger (1970) exposed western white suckers to water containing ^{14}C-labelled endosulfan at 29 μg L^{-1} for 12 h. In the tissues concentrating the most endosulfan, a peak level of the compound was achieved within 12 h. A plateau was maintained over a prolonged period in studies on goldfish exposed to endosulfan solutions at concentration of 7 μg L^{-1}. Residue levels in muscle were 2.54 mg kg^{-1} after 5 days and 1.09 mg kg^{-1} after 20 days (Schoettger 1970). Accumulation appeared to be transitory, because endosulfan disappeared rapidly in the mussels (Roberts 1972) and goldfish after the source was removed (Schoettger 1970).

α-endosulfan is more bioaccumulative than the β-isomer or the sulphate. The US EPA (2002) considered endosulfan to have a high potential to bioaccumulate in fish, and hence may affect animals higher in the food chain. DeLorenzo et al. (2002) noted that the BCF of 3278 for *Daphnia* is similar to those reported for the POPs pesticides DDT, dieldrin and chlordane of 2500–12,000 ug/l. They also noted that BCF of 3278 was for neonates and it is expected to be higher for adult *Daphnia*, which have nearly five times higher lipid content.

Landers et al. (2008) analysed airborne pollutants in the western national parks of the USA, from 2002 to 2007 and found an evidence of the bioaccumulation of endosulfan in vegetation. The level of accumulation increased with forest productivity and proximity to sources of endosulfan. The mean level of total endosulfan in two-year-old needles of White Fir (7573 ug kg^{-1} lipid) was 3 times higher than the levels in one-year-old needles (2448 ug kg^{-1}). The degree of bioaccumulation varies among species with firs accumulating substantially higher concentrations than pines. The coniferous forests of the western USA and Alaska are estimated to be removing between 1280 and 7210 kg of endosulfan from the air annually, amounting to about 2 % of the USA's annual usage. Menone et al. (2000) found residues of endosulfan sulphate in the liver, gonads, fat and muscle of fish from a coastal lagoon in Argentina, up to 0.018 ug kg^{-1} of tissue. They calculated a BMF of 4.02, higher than that for DDT.

3.5 Fate of Endosulfan in Environment

Like all other pesticides, endosulfan, once enters the environment, can undergo several process which eventually decides its persistence and mobility. The fate of endosulfan in the environment is different for its two isomers, β-endosulfan being

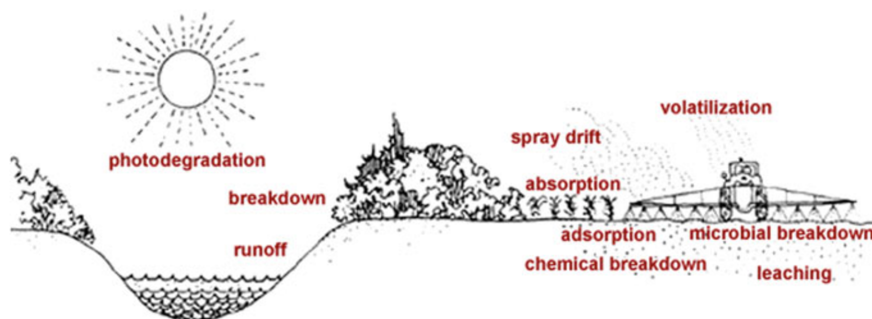


Fig. 1 Fate of endosulfan in the environment

more persistent than α-isomer (Siddique et al. 2003) and also depends on the medium, it gets deposited. The overall dissipation of endosulfan from the environment depends on various processes viz. degradation, adsorption, volatilization, leaching, absorption etc. The intensity of these processes determines endosulfan fate in the particular environment (Fig. 1).

3.5.1 Adsorption

The binding of pesticide to soil particles depends on nature of pesticide, soil, moisture, pH and soil texture. It determines the bioactivity, leachability, bioavailability and degradability of the pesticide in soil. Adsorption of endosulfan is directly proportional to the particle size of soil β-endosulfan is generally more strongly adsorbed than α-endosulfan. High adsorption capability of endosulfan makes it highly persistent in soil with half life of about 60–800 days as compared to 35–150 days in water (Guerin 2001).

3.5.2 Volatilization

Volatilization of a compound depends on physico-chemical properties of the pesticide, climatic condition and mode of applications. In general, pesticides are volatilized more from the plant surface than the soil which can lead to air pollution and transport pesticide to a far off place.

Since endosulfan is a semi-volatile compound; it can evaporate from soil, plant surface or even water (Guerin and Kennedy 1992; Guerin 2001). Laboratory studies at 22 °C at relative humidity of 50 % found: (a) 25–30 % dissipation from soil surface in 24 h, (b) 64 % dissipation from beans leaves and (c) α-endosulfan is more volatile than β-isomer, endosulfan-sulphate the least (GFEA-U. 2007).

3.5.3 Runoff

Runoff is the movement of pesticide in water over a sloping surface either mixed with water or bound to eroding soil. This is a major concern for environmentalists, as it can lead to contamination of surrounding water body. Pesticide runoff becomes important particularly during the period of intense rainfall. Since endosulfan binds strongly to the soil particles, there is always a considerable chance of runoff and danger of contamination due to erosion of the soil.

3.5.4 Leaching

It is the movement of pesticide with water through soil—downward, upward or sideways. It occurs mainly through moving water into the soil matrix and through macro-pores. Therefore solubility of pesticide is a major factor for leaching. Endosulfan being insoluble in water has a very little chance of leaching, however, reports of groundwater contamination is reported from many parts of India (Kumari et al. 2008), US (USEPA 2002), Portugal (Goncalves et al. 2007), South Africa (Dalvie et al. 2003) and Morocco (El Bakouri et al. 2007).

3.5.5 Degradation of Endosulfan

Degradation is the process of pesticide breakdown after application. Pesticides can be broken down by microbes, chemical reactions, and/or light. Its degradation depends on environmental conditions and chemical characteristic of the pesticide. These factors determine the actual persistence of the pesticide in the environment. Pesticides, that break down quickly generally, do not persist in the environment or on the crop. However, pesticides that break down rapidly may only provide short-term control and ineffective for the actual purpose of pest control (Arbeli and Fuentes 2007).

In the environment, endosulfan can undergo three different types of degradation process: (a) Microbial degradation, (b) Chemical degradation and (c) Photodegradation.

Microbial Degradation of Endosulfan

Microorganisms play a very important role in removing pesticides from the contaminated sites. Literally, there are no such compounds which these microbes cannot degrade. The ability of these microorganisms to degrade a large variety of compounds is mainly attributed to their dynamic, complex and complicated enzymatic systems. Owing to their diverse metabolic activities, extensive modification in the structure and toxicological properties of the contaminants take place

which ultimately results in the complete conversion of any pesticide into simple by-products. The ability of microbes to degrade pesticides at a much faster rate makes them suitable for bioremediation techniques.

Microorganisms gain energy by catalyzing energy-producing chemical reactions that involve breaking chemical bonds and transferring electrons from the contaminant. The type of chemical reaction is called an oxidation-reduction reaction: the organic contaminant is oxidized or reduced. The contaminant is called the electron donor, while the electron recipient is called the electron acceptor. The energy gained from these electron transfers is then "invested," along with some electrons and carbon from the contaminant is used to produce more cells.

Other than the oxidation-reduction reaction, microbial degradation or metabolism also involves hydrolysis reaction. Endosulfan can undergo both oxidation and hydrolysis reaction to form toxic endosulfan-sulphate and non-toxic endosulfan-diol respectively. Endosulfan sulphate is exclusively formed by microbial oxidation while endosulfan-diol can be formed by chemical hydrolysis of endosulfan (Martens 1976). Thus, microorganism can use endosulfan either as a sole source of sulphur or carbon. Some bacteria are also able to use endosulfan as source of both sulphur and carbon (Fig. 2).

a. **Endosulfan as a Sulphur Source**

Sulphur is an essential element for all living cell. It is needed for the synthesis of substances, like the amino acids (cysteine and methionine) and some carbohydrates (biotin, and thiamine). Besides, it is an essential component of cytochrome c oxidase which is involved in the aerobic respiration in all cells. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulphate reduction. However, a few require a reduced form of sulfur, such as cysteine.

In the laboratory, bacteria can be grown in a sulphur free medium with endosulfan as the sole source of sulphur (Sutherland et al. 2000). Endosulfan has a relatively reactive cyclic sulfite diester group (Van Woerden 1963). Microorganisms, with the ability to release the sulfite group from endosulfan use it as a source of sulphur for their growth. Therefore, simple initial enrichment technique in a sulphur free medium with endosulfan as sole source of sulphur will encourage only those microorganisms capable of utilizing endosulfan. Further enrichment can lead to artificial selection of only those microorganisms capable of degrading/detoxifying endosulfan. Since the removal of the sulphur moiety decreases vertebrate toxicity, (Dorough et al. 1978; Goebel et al. 1982), this method uses organisms capable of detoxifying the insecticide.

Sutherland et al. (2000) isolated endosulfan degrading bacteria from soil through selective enrichment technique using endosulfan as sole source of sulphur. The isolated mixed bacterial culture was able to grow and degrade endosulfan in the sulphur free medium. Degradation of endosulfan occurs in concomitant with bacterial growth. Hussain et al. (2007) isolated 29 bacterial strains through enrichment technique. These strains differed substantially in their potential to degrade endosulfan ranging from 40 to 93 % in in vitro conditions.

Fig. 2 Microbial metabolism of endosulfan (Klema and LaBelle 2007)

Wen Li et al. (2008) isolated *Achromobacter xylosoxidans* strain CS5 using selective enrichment technique in sulphur free medium. This strain was able to degrade 24.8 and 10.5 mg l^{-1} of α- and β-endosulfan, respectively, in 8 days. The major metabolites formed were endosulfan diol and endosulfan ether. The cell-free extract of the same strain grown in non-endosulfan medium was also able to degrade endosulfan very rapidly, showing that the degradative enzymes were expressed constitutively in this bacterial strain.

b. **Endosulfan as Carbon Source**

Many bacteria and other microorganism are capable of utilizing endosulfan as carbon and energy source. Although endosulfan is regarded as poor biological energy source (Guerin 1999), but many researchers have been able to isolate bacteria capable of using endosulfan as carbon source through the selective enrichment technique.

Siddique et al. (2003) isolated bacterial strains capable of degrading up to 81.8 % α-endosulfan and 86.8 % β-endosulfan through enrichment with endosulfan as sole source of carbon. Awasthi et al. (1997) isolated bacterial co-culture through enrichment technique which can degrade endosulfan and grow in carbon free medium. Likewise, Kumar and Philip (2006) isolated three bacteria such as *Staphylococcus* sp. *Bacillus circulans-I* and *Bacillus circulans II*. These bacteria were capable of degrading 71.82 and 76.04 % of spiked endosulfan in aerobic and anaerobic condition, respectively. Addition of auxiliary carbon source i.e. dextrose, enhances the degradation efficiency by 13.36 and 12.33 % in aerobic and anaerobic condition, respectively.

c. **Enzymatic Degradation of Endosulfan**

Enzymes are protein synthesized by living organisms to be used in transforming a specific compound. It serves as a catalyst in the biochemical transformation. The microorganisms, which break down toxicants in contaminated soil or water, can be sources of enzymes that detoxify pesticide residues. The application of these enzymes is particularly suitable for pesticide contaminated water, as they cause rapid remediation without the addition of nutrients or aeration (Sutherland et al. 2002).

The process of enzymatic bioremediation involves the following four steps:

- Source of enzyme is identified in bacteria or from other natural sources
- The gene encoding the enzyme is identified and isolated
- The enzyme is cloned into a common bacterium, such as *Escherischia coli*
- The enzyme is produced by industrial-scale fermentation of the *E. coli*. Once there is a sufficient volume, the *E. coli* is killed and the enzymes, which have been produced by the bacterium, are collected and applied to the contaminated water (Sutherland et al. 2002).

CSIRO Entomology, in conjunction with members of the Advanced Water Technologies business of Orica Australia Pty Ltd. and CSIRO Molecular Sciences, has successfully developed enzyme-based bioremediation technologies for detoxifying various pesticides in contaminated water. In their first field trial, methyl parathion levels in 80,000 L of fast flowing run-off water in cotton farm drainage channels were reduced by 90 % in less than 10 min.

Weir et al. (2006) isolated a gene, *ese*, encoding an enzyme capable of degrading both isomers of endosulfan and endosulfan sulphate an *Arthrobacter* species. They characterised the enzyme which belongs to the two-component flavin-dependent

monooxygenase family whose members require reduced flavin for activity. The enzyme catalysed the degradation of endosulfan to endosulfan monoalcohol and endosulfan sulphate to hemisulfate.

d. **Effect of Environmental Factors on Biodegradation of Endosulfan**

Since endosulfan is a chemical compound, its degradation in the environment is affected by all those factors which affect the stability of the compound as well as growth of micro-organism viz, temperature, pH, soil texture, organic matter content, moisture, redox potential etc. These factors vary with time and space; hence it is important to determine the optimum environmental condition in order to carry out successful bioremediation process. Because, there have been many reports of failure of bioremediation when the laboratory grown microorganisms are applied in the field for this purpose.

Degradation of endosulfan is, in fact, favoured by environmental conditions that encourage the chemical hydrolysis of the compound and growth of microbial population. Under similar moisture and temperature, it was found that degradation of endosulfan was more in soil with higher organic contents. Arshad et al. (2007) studied the degradation of endosulfan under different environmental conditions by *Pseudomonas aeruginosa* and concluded that degradation was most effective at incubation temperature of 30 °C and aerated slurries soil having alkaline pH of 8. Degradation was more rapid in coarse textured soil than fine textured soil. It was also observed that degradation of endosulfan was minimum in acidic pH (5 pH) and increased with an increase in pH up to 8 pH.

Chemical Degradation of Endosulfan

Chemical degradation or abiotic degradation of pesticide usually occurs by different chemical reaction viz., hydrolysis, oxidation-reduction and ionisation. The type and rate of chemical reactions depend on the properties of pesticide, temperature, pH and moisture. As endosulfan does not contain any oxidising molecules, it has no oxidising properties. Moreover, due to its molecular structure, the substance cannot dissociate and possess no acid protons or an appreciable number of basic centres. However, endosulfan is sensitive to acids, alkalis and moisture and subjected to slow hydrolysis to endosulfan diol and sulphur dioxide in the alkali medium (Martens 1976).

a. **Chemical Hydrolysis of Endosulfan**

Guerin (2001) studied the abiotic loss of endosulfan in presence and absence of oxygen and found that half life of α-endosulfan increased substantially in absence of oxygen i.e. in sealed oxygen-limited vessels; while under aerobic condition, β-endosulfan seems to be far more persistent. Thus, he concluded that α-endosulfan being more volatile than β-endosulfan is subjected to faster dissipation in unsealed aerobic condition. Moreover, endosulfan sulphate, the major metabolite of

endosulfan in the environment, was not formed in any of the experiment, suggesting that oxidation of endosulfan to endosulfan sulphate is exclusively carried out by the microbial oxidising enzyme, while endosulfan diol is the major product formed in chemical hydrolysis of endosulfan.

Photodegradation

Photodegradation is the breakdown of pesticide by light (sunlight) and can occur on foliage, on surface of soil and in air. It occurs when radiant energy in form of photons breaks the chemical bonds of the molecule. The rate of photochemical reaction depends on bond energy i.e. energy required to break the chemical bond, available light intensity and the presence of intermediate compounds making indirect photolysis possible. All pesticides are susceptible to photodegradation to some extent. Factors affecting pesticide photodegradation are intensity of the sunlight, exposure time, properties of sites, method of application and properties of the pesticide.

Endosulfan is relatively stable to light in visible spectrum. However, it is subjected to photochemical degradation under ultra-violet light, forming endosulfan diol. The degradation pathway involves formation of endosulfan diol, its transformation to endosulfan ether and finally ether complete degradation. The photochemical degradation of endosulfan follows first order kinetics (Barcelo-Quintal et al. 2008).

3.5.6 Fate of Endosulfan in Mammals

Endosulfan is rapidly degraded into mainly water-soluble compounds and eliminated in mammals with very little absorption in the gastrointestinal tract. Endosulfan has also been detected from cord blood samples obtained at the time of delivery (Cooper et al. 2001), human sera (Martinez et al. 2000; Younglai et al. 2002), adipose tissue (Amaraneni and Pillale 2001) and human milk samples obtained from healthy lactating women in Spain (Campoy et al. 2001). Residues were also detected in fat samples from children living nearby farms in Spain (OleaSerrano et al. 1999). Blood, human milk and urine samples in Croatia were also found to be contaminated with endosulfan (Fig. 3).

In rabbits, the β-isomer is cleared from blood plasma more quickly than the α-isomer, with reported blood half-lives of approximately 6 h and 10 days, respectively. The metabolites are dependent on the mixture of isomers and the route of exposure (Rajendran and Venugopalan 1991). Most of endosulfan seems to leave the body within a few days to a few weeks. It is also toxic to mammals, like rabbits (Sang and Petrovic 1999; Thanal Conservation Action and Information Network 2001) and rats (Reuber 1981; Kalendar et al. 2004). The disappearance of cats, frogs, bees, fresh water fishes etc. were reported form Kasaragod District of Kerala in South India, where endosulfan was aerially sprayed for over 20 years (Thanal

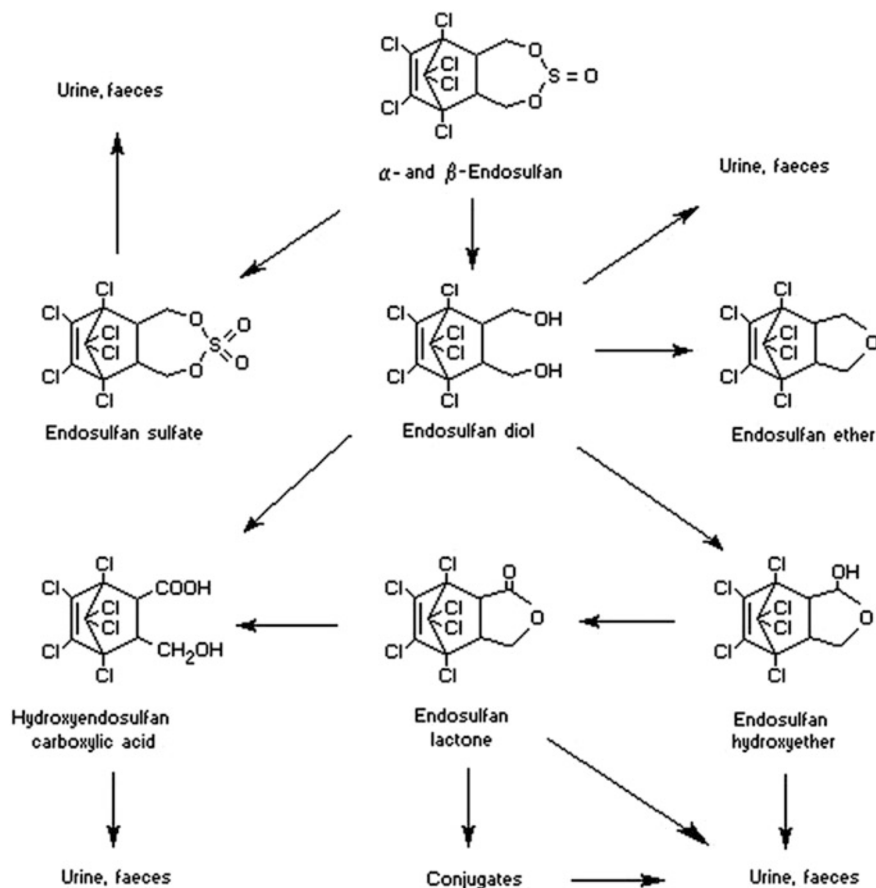


Fig. 3 Mammalian metabolism and excretion of endosulfan (McGregor 1998)

Conservation Action and Information Network 2001). Endosulfan is also highly poisonous to cats (LD_{50}-2 mg kg^{-1}) and dogs (LD_{50}-76.7 mg kg^{-1}). Reversible blindness and lack of muscle coordination have been reported in sheep and cattle grazing on endosulfan contaminated grass (PAN-UPS 1996). Chronic exposure to endosulfan leads to deleterious effects on metabolism and immune system of broiler chicken (Garg et al. 2004).

3.6 Present Regulatory Status of Endosulfan

Endosulfan has been in world-wide use since its introduction in the 1950s. It was considered a safer alternative to other organochlorine pesticides in many countries

all over the world since the 1970s. But in the last two decades many countries have recognized the hazards of wide application of this pesticide. As the results, either they have stopped its production and/or banned or restricted its use. Endosulfan is banned in countries like Singapore, Belize, Tonga, Syria, Germany, Sweden, Philippines, Netherlands, St. Lucia, Columbia, Cambodia, Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, UAE, Sri Lanka and Pakistan. Restricted use is allowed in Australia, Bangladesh, Indonesia, Iran, Japan, Korea, Kazakhstan, Lithuania, Thailand, Taiwan, Denmark, Serbia & Montenegro, Norway, Finland, Russia, Venezuela, Dominican Republic, Honduras, Panama, Iceland, Canada, US and UK.

It is one among the twenty one priority compounds identified by the UNEP-GEF in the Regional Based Assessment of Persistent Toxic Substances (PTS), 2002. These reports have taken into account the magnitude of use, environmental levels and human and ecological effects of these compounds. In the Indian Ocean Region, endosulfan is banned in 8 nations. India is one of the major Indian Ocean rim nations, which has imposed no ban or restrictions on endosulfan. A ban on endosulfan exists in the South Indian state of Kerala, which came as a result of a public pressure following the poisoning of many villages due to aerial spraying of this chemical. Colombia and Cambodia are two countries where endosulfan was banned from use recently. In the Reports of the Regional Based Assessment of PTS of the UNEP- GEF, endosulfan has been rated depending on the level of concern in the respective region countries. They are as follows:

- **Indian Ocean region**—"Regional Concern"
- **North American region**—Treated as "Regionally specific PTS", receiving great attention along with HCH, Chlordane, PCB, PAHs.
- **Mediterranean region**—"Local Concern".
- **Sub Saharan Africa**—Ranked as the PTS of highest concern after DDT.
- **Eastern and Western South America**—"Potentially relevant PTS of emerging concern".
- **European region**—"Proposed Possible Priority Hazardous Substance".
- **South East Asia and Southern Pacific region**—"Regional Concern". It is identified as a major PTS which has a continuing effect on the natural ecosystem in the region and long term effect on the structure of aquatic eco system.
- **Central America and the Caribbean**—Considered as one of most important PTS of emerging concern.

In October, the Review Committee of the Stockholm Convention moved endosulfan in the procedure for listing under the treaty, while India blocked its addition to the Rotterdam Convention. Recently, endosulfan was banned in New Zealand by the Environmental Risk Management Authority effective January 2009 after a concerted campaign by environmental groups and the Green Party.

4 Conclusions and Future Perspectives

Bioremediation has several limitations. It depends on the nature of organisms, the enzyme involved, its concentration and availability and finally survival of microorganisms. If an appropriate microorganism is absent in soil or if biodegrading microbial population is reduced due to toxicity of pesticide, a specific microorganism can be added or introduced in soil to enhance the activity of the existing population. This microorganism is either natural or genetically engineered. "Superbugs" are also developed that can degrade the pesticide at fast rate. Therefore, to some extent, these problems can be solved by understanding the genetics and biochemistry of desired microbe.

Degradation is catalysed by specific enzyme produced by degrading cell or enzyme found external to the cell. Degradation of pesticide by either external or internal enzyme will stop at any step, if an appropriate enzyme is not present. Absence of an appropriate enzyme is one of the common reasons for persistence of any pesticide. Therefore, sometimes the use of microbial enzyme directly to the pollutant may bypass these constrains associated with live cells. Hence, the use of enzymes for degradation of pesticides can be developed as a future technology for bioremediation.

References

Amaraneni, S. R., & Pillale, R. R. (2001). Concentrations of pesticide residues in tissues of fish from Kolleru lake in India. *Environmental Toxicology, 16*, 550–556.

Anonymous. (1997). Existing chemical review programme—NRA review of endosulfan—Draft National Registration Authority for Agriculture and Vetenary Chemicals.

Anonymous. (2002). Regional based assessment of persistent toxic substances—Arctic regional report-chemicals-united Nations Environmental Programme-Global Environment Facility.

Arbeli, Z., & Fuentes, C. L. (2007). Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. *Crop Protection, 26*, 1733–1746.

Arshad, M., Hussain, S., & Saleem, M. (2007). Optimization of environmental parameters for biodegradation of alpha and beta endosulfan in soil slurry by Pseudomonas aeruginosa. *Journal of Applied Microbiology*, *104*, 364–370.

ATSDR. (2000). *Toxicological profile for endosulfan. Agency of toxic substances and disease registry*. Atlanta, USA. http://www.atsdr.cdc.gov/toxprofiles/tp41.html

Awasthi, N., Manickam, N., & Kumar, A. (1997). Biodegradation of endosulfan by a bacterial coculture. *Bulletin of Environment Contamination and Toxicology, 59*, 928–934.

Barcelo-Quintal, M. H., Cebada-Ricalde, M. C. C. R., Angel, R. T. I., Reyna, B. R. O., & Jose, A. M. C. (2008). Kinetic studies of endosulfan photochemical degradations by ultraviolet light irradiation in aqueous medium. *Journal Environment Science Health, 43*, 120–126.

Bhattacharya, S., Gosh, R. K., Mandal, T. K., Chakraborty, A. K., & Basak, D. K. (1993). Some histological changes in chronic endosulfan (Thionol) toxicity in poultry. *Indian Journal Animal Health, 32*, 9–11.

Broomhall, S. (2002). The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles. *Aquatic Toxicol, 61*, 243–250.

Broomhall, S., & Shine, R. (2003). Effects of the insecticide endosulfan and presence of congeneric tadpoles on Australian tree frog (*Litoria freycineti*) tadpoles. *Archives of Environmental Contamination and Toxicology, 45*(2), 221–226.

Campoy, C., Jimenez, M., Olea Serrano, M. F., Olea, N., Moreno, F. M., Canabate, F., et al. (2001). Analysis of organochlorine pesticides in human milk: preliminary results. *Early Human Develop, S65*, 183–190.

Chowdhari, D. K., Nazir, A., & Saxena, D. K. (2001). Effect of Three Chlorinated Pesticides on hsr ω stress gene in transgenic *Drosophila melanogaster*. *Journal Biochemical Mole Toxicology, 15*, 173–186.

Cooper, S. P., Burau, K., Smith, M. A., Symanski, E., Sweeny, A., Robison, T., et al. (2001). Prenatal exposure to pesticides-a feasibility study among migrant and seasonal workers. *American Journal Industrial Medicine, 40*, 578–585.

Dalvie, M. A., Cairncross, E., Solomon, A., & London, L. (2003). Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. *Environment Health, 2*(1), 1–15.

Daly, H., Doyen, J. T., & Purcell, A. H, I. I. I. (1998). *Introduction to insect biology and diversity* (2nd ed., pp. 279–300). New York: Oxford University Press.

Daniel, C. S., Agarwal, S., & Agarwal, S. S. (1986). Human red blood cell membrane damage by Endosulfan. *Gene Appl Entomol Toxicol Lett, 32*, 113–118.

DeLorenzo, M. E., Taylor, L. A., Lund, S. A., Pennington, P. L., Strozier, E. D., & Fulton, M. H. (2002). Toxicity and bioconcentration potential of the agricultural pesticide endosulfan in phytoplankton and zooplankton. *Archives of Environmental Contamination and Toxicology, 42*, 173–181.

Dorough, H. W., Huhtanen, K., Marshall, T. C., & Bryant, H. E. (1978). Fate of endosulfan in rats and toxicological considerations of apolar metabolites. *Pesticide Biochemistry Physiology, 8*, 241–252.

Dutta, H. M., & Arends, D. A. (2003). Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. *Environmental Research, 91*(3), 157–162.

El Bakouri, H., Ouassini, A., Morillo Aguado, J., & Usero García, J. (2007). Endosulfan sulfate mobility in soil columns and of groundwater in Northwest Morocco. *Water Environment Research, 79*(13), 2578–2584.

Garg, U. K., Pal, A. K., Jha, G. J., & Jadhao, S. B. (2004). Haemato-biochemical and immuno-pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides in broiler chicks. *International Immunopharmacology, 4*(13), 1709–1722.

GFEA-U. (2007). *Endosulfan, draft dossier prepared in support of a proposal of endosulfan to be considered as a candidate for inclusion in the CLRTAP protocol on persistent organic pollutants*. German Federal Environment Agency—Umweltbundesamt, Berlin

Goebel, H., Gorbanch, S., & Khauf, W. (1982). Properties, effects, residues, and analytics of the insecicide endosulfan. *Residue Rev, 83*, 1–100.

Goncalves, C. M., Silva, J. C., & Alpendurada, M. F. (2007). Evaluation of the pesticide contamination of groundwater sampled over two years from a vulnerable zone in Portugal. *Journal of Agriculture and Food Chemistry, 55*(15), 6227–6235.

Guerin, T. F. (1999). The anaerobic degradation of endosulfan by indigenous microorganisms from low-oxygen soils and sediments. *Environmental Pollution, 106*, 13–21.

Guerin, T. F. (2001). Abiological loss of endosulfan and related chlorinated organic compounds from aqueous systems in the presence and absence of oxygen. *Environmental Pollution, 115*, 219–230.

Guerin, T. F., & Kennedy, I. R. (1992). Distribution and dissipation of endosulfan and related cyclodienes in sterile aqueous systems: Implications for studies on biodegradation. *Journal of Agriculture and Food Chemistry, 40*, 2315–2323.

Hodgson, E. (2004). *A textbook of modern toxicology*. Hoboken, New Jersey: Wiley.
Hurt, S. S. (1991). Dicofol: Toxicological evaluation of dicofol prepared for the WHO expert group on pesticide residues (Report no. 91 R-1017). 6:6.
Hussain, S., Arshad, M., Saleem, M., & Khalid, A. (2007). Biodegradation of α- and β-endosulfan by soil bacteria. *Biodegradation, 18*, 731–740.
IPCS. (1984). Environmental health criteria 40. endosulfan. International Programme on Chemical Safety, World Health Organisation, Geneva. Online at http://www.inchem.org/documents/ehc/ehc/ehc40.htm
Jayashree, R., & Vasudevan, N. (2007). Persistence and distribution of endosulfan under field conditions. *Environmental Monitoring and Assessment, 131*(1–3), 475–487.
Jin, L., Tran, D. Q., Ide, C. F., McLachlan, J. A., & Arnold, S. F. (1997). Several synthetic chemicals inhibit progesterone receptor-mediated trans-activation in yeast. *Biochemical Biophysics Research Communications, 233*, 139–146.
JMPR. (2008). http://www.who.int/foodsafety/publications/jmpr-reports/en/
Joshi, S. (2001). Children of Endosulfan. Down to earth, 19: 28.
Kalendar, S., Kalendar, Y., Ogutcu, A., Uzunhisarcikli, M., Durak, D., & Acikgoz, F. (2004). Endosulfan-induced cardio-toxicity and free radical metabolism in rats- the protective effect of Vitamin E. *Toxicology, 202*, 227–235.
Kelly, B. C., Ikonomou, M. G., Blair, J. D., Morin, A. E., & Gobas, F. A. P. C. (2007). Food web-specific biomagnification of persistent organic pollutants. *Science, 317*, 236–239.
Klema, V., & LaBelle, E. (2007). Endosulfan pathway map. Online at http://umbbd.msi.umn.edu/end/end_map.html
Kumar, M., & Philip, L. (2006). Endosulfan mineralization by bacterial isolates and possible degradation pathway identification. *Bioremediation Journal, 10*(4), 179–190.
Kumari, B., Madan, V. K., & Kathpal, T. S. (2008). Status of insecticide contamination of soil and water in Haryana, India. *Environmental Monitoring and Assessment, 136*(1–3), 239–244.
Kurkure, N. V., Bhandarkar, A. G., Joshi, M. V., Sadekar, R. D., & Bhagwat, S. S. (1993). Immunosuppressive and histotoxic effects of endosulfan in chicks. *Indian Journal Animal Science, 63*, 1258–1260.
Landers, D. H., Simonich, S. L., Jaffe, D. A., Geiser, L. H., Campbell, D. H., Schwindt, A. R., et al. (2008). The fate, transport, and ecological impacts of airborne contaminants in western national Parks (USA). EPA/600/R-07/138. U.S. Environmental Protection Agency, Office of Research and Development, NHEERL, Western Ecology Division, Corvallis, Oregon.
Li, Y. F., & Macdonald, R. W. (2005). Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: a review. *Science of the Total Environment, 342*, 87–106.
Li, J., Zhang, G., Guo, L., Xu, W., Li, X., Lee, C. S. L., et al. (2007). Organochlorine pesticides in the atmosphere of Guangzhou and Hong Kong: Regional sources and long-range atmospheric transport. *Atmospheric Environment, 41*, 3889–3903.
Li, W., Yun, D., Beibei, X., Li, Y., Xiang, P., Jingshun, Z., et al. (2008). Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5. *Journal of Hazardous Materials, 167*, 209–216.
Lu, Y., Morimoto, K., Takeshita, T., Takeuchi, T., & Saito, T. (2000). Genotoxic effects of alpha-endosulfan and beta-endosulfan on human HepG2 cells. *Environmental Health Perspectives, 108*(6), 559–561.
Martens, R. (1976). Degradation of [8, 9-^{14}C] endosulfan by soil microorganisms. *Applied and Environment Microbiology, 6*, 853–858.
Martinez, V. J. L., Moreno, F. M., Garrido, F. A., & Olea Serrano Olea, N. (2000). Determination of endocrine-disrupting pesticides and polychlorinated biphenyls in human serum by GC-ECD and GC-MS-MS and evaluation of contribution to the uncertainity of results. *Analytical and Bioanalytical Chemistry, 372*, 766–775.
McGregor, D. B. (1998). *Endosulfan—The joint FAO/WHO meeting on pesticide residues (JMPR)*. France: International Agency for Research on Cancer Lyon.

Menone, M. L., Aizpun de Moreno, J. E., Moreno, V. J., Lanfranchi, A. L., Metcalfe, T. L., & Metcalfe, C. D. (2000). PCBs and organochlorines in tissues of silverside (*Odontesthes bonariensis*) from a coastal lagoon in Argentina. *Archives of Environmental Contamination and Toxicology, 38*, 202–208.

Naqvi, S. M., & Vaishnavi, C. (1993). Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. *Comparative Biochemistry and Physiology, 105C*, 347–361.

Neuparth, T., Bickham, J. W., Theodorakis, C. W., Costa, F. O., & Costa, M. H. (2006). Endosulfan-induced genotoxicity detected in the Gilthead Seabream, *Sparus aurata* L., by means of flow cytometry and micronuclei assays. *Bulletin of Environment Contamination and Toxicology, 76*(2), 242–248.

NIOH. (2003). *Final report of the investigation of unusual illnesses allegedly produced by endosulfan exposure in Padre village of Kasargod District (N Kerala)*. Ahmedabad: National Institute of Occupational Health. Indian Council for Medical Research.

Oerke, E. C., & Dehne, H. W. (2004). Safeguarding production-losses in major crops and the role of crop protection. *Crop Protection, 23*, 275–285.

OleaSerrano, F., Olea, N., LardelliClaret, P., Rivas, A., & BarbaNavarro, A. (1999). Inadvertant exposure to xenoestrogens in children. *Toxicology Industrial Health, 15*, 151–158.

Pal, A. K., Prasad, R. L., Prasad, A., Singh, S. K., Kumar, A., & Jadhao, S. B. (2005). In ovo-embryotoxicity of a-endosulfan adversely influences liver and brain metabolism and the immune system in chickens. *Pest Biochemical Physiology, 82*, 103–114.

Palma, P., Palma, V. L., Fernandes, R. M., Soares, A. M. V. M., & Barbosa, I. R. (2009). Endosulfan sulphate interferes with reproduction, embryonic development and sex differentiation in *Daphnia magma*. *Ecotoxicology Environment Safety, 72*, 344–350.

Pandey, N., Gundevia, F., Prem, A. S., & Ray, P. K. (1990). Studies on the genotoxicity of endosulfan, an organochlorine insecticide, in mammalian germ cells. *Mutation Research, 242* (1), 1–7.

Pandey, S., Nagpure, N. S., Kumar, R., Sharma, S., Srivastava, S. K., & Verma, M. S. (2006). Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single-cell gel electrophoresis. *Ecotoxicology Environment Safety, 65*(1), 56–61.

PAN-UPS. (1996). Endosulfan responsible for Alabama fish killed. *Pesticide action network, North America updates service*.

Park, D., Hempleman, S. C., & Propper, C. R. (2001). Endosulfan exposure disrupts pheromonal systems in the red-spotted newt: a mechanism for subtle effects of environmental chemicals. *Environmental Health Perspectives, 109*, 669–673.

Park, D., & Propper, C. R. (2002). Endosulfan affects pheromonal Detection and glands in the male red-spotted newt, *Notophthalmus viridescens*. *Bulletin of Environment Contamination and Toxicology, 69*, 609–616.

Paul, V., Balasubrahmaniam, E., Jayakumar, A. R., & Kazi, M. (1995). A sex related difference in the neuro behavioural and hepatic effects following chronic endosulfan treatment in rats. *European Journal Pharmacology, 293*, 355–360.

Peshin, R. (2002). Economic benefits of pest management. In D. Pimental (Ed.), *Encyclopedia of pest management* (pp. 224). Marcel Dekker, Inc.

Pesticide Action Network. (2008). Thousands tell EPA: Phase out endosulfan. Online at http://www.panna.org/newsroom/20080218

Preziosi, P. (1998). Naturo and anthropogenic environmental estrogens—The scientific basis for risk assessment, endocrine-disruptors as environmental signalers—An introduction. *Pure and Applied Chemistry, 70*, 1617–1631.

Qaim, M., & Zilberman, D. (2003). Yield effects of genetically modified crops in developing countries. *Science, 299*, 900–902.

Quijano, R. F. (2000). Risk assessment in a third world reality: An endosulfan case history. *International Journal Occupation Environmental Health, 6*(4), 312–317.

Quijano, R. F. (2002). *Endosulfan poisoning in Kasargod, Kerala, India: Report on a fact-finding mission*. Penang: Pesticide Action Network Asia and the Pacific.

Rajendran, N., & Venugopalan, K. V. (1991). Bioconcentration of endosulfan in different body tissues of estuarine organisms under sublethal exposure. *Bulletin of Environment Contamination and Toxicology, 46*, 151–158.
Reuber, M. D. (1981). The role of toxicity in the carcinogenicity of Endosulfan. *Science of the Total Environment, 20*, 23–47.
Roberts, D. (1972). The assimilation and chronic effects of sub-lethal concentrations of endosulfan on condition and spawning in the common mussel *(Mytilus edulis)*. *Marine Biology, 16*, 118–125.
Saiyed, H., Dewan, A., Bhatnagar, V., Shenoy, U., Shenoy, R., Rajmohan, H., et al. (2003). Effect of endosulfan on male reproductive development. *Children's Health Article, 111*, 16.
Sang, S., Petrovic, S. (1999). Endosulfan-a review of its toxicity and its effects on the endocrine system WWF.
Schoettger, R. A. (1970). Toxicology of thiodan in several fish and aquatic invertebrates. *US Department of the Interior, Bureau of Sport, Fish and Wildlife, Investigations in Fish Control, 35*, 1–31.
Sharma, S., Nagpure, N. S., Kumar, R., Pandey, S., Srivastava, S. K., Singh, P. J., et al. (2007). Studies on the genotoxicity of endosulfan in different tissues of fresh water fish *Mystus vittatus* using the comet assay. *Archives of Environmental Contamination and Toxicology, 53*, 617–623.
Siddique, T., Okeke, B. C., Arshad, M., & Frankenberger, W. T, Jr. (2003). Enrichment and isolation of endosulfan-degrading microorganisms. *Journal of Environmental Quality, 32*, 47–54.
Sinha, N., Narayan, R., Sankar, R., & Saxena, D. K. (1995). Endosulfan induced bio chemical changes in testis of rats. *Veterinary Human Toxicol, 37*, 547–549.
Smith, A. G. (1991). Chlorinated hydrocarbon insecticides. *In Handbook of Pesticide Toxicology, 6*, 3.
Sobti, R. C. (1983). Cytokinetic and cytogenetic effect of agriculture chemicals on human lymphoid cells in vitro. II. Organochlorine pesticides. *Archieves Toxicology, 52*, 221–231.
Soto, A. M., Chung, K. L., & Sonnen, S. C. (1994). The pesticides endosulfan, toxaphene and dieldrin have estrogenic effect on human estrogen—sensitive cells. *Environmental Health Perspectives, 102*, 380–383.
Sutherland, T. D., Horne, I., Lacey, M. J., Harcourt, R. L., Russell, R. J., & Oakeshott, J. G. (2000). Enrichment of an endosulfan-degrading mixed bacterial culture. *Applied and Environment Microbiology, 66*, 2822–2828.
Sutherland, T. D., Robyn, R., & Selleck, M. (2002). Using enzymes to clean up pesticide residues. *Pesticide Outlook*, 149–151
Thanal Conservation Action and Information Network. (2001). India preliminary findings of the survey on the impact of aerial spraying on the people and the ecosystem: Long term monitoring —The impact of pesticides on the people and ecosystem (LMIPPE).
Ton, P., Tovignan, S., & Davo, S. (2000). Endosulfan deaths and poisonings in Benin. *Pesticides News, 47*, 12–14.
UNEP/FAO. (2007). *Inclusion of the chemical endosulfan in Annex III of the Rotterdam convention*. United Nations Environment Programme and Food and Agriculture Organisation UNEP/FAO/RC/COP.4/9. http://www.pic.int/COPS/COP4/i9)/English/K0763331%20COP-4-9.pdf
US Agency for Toxic Substances and Disease Registry. (1990). Toxicological profile for endosulfan. *Draft Report, 6*:52.
USEPA. (2002). *Reregistration eligibility decision for endosulfan. EPA 738-R-02-013. Pollution, pesticides and toxic substances (7508C)*. United States Environmental Protection Agency. Online at http://www.epa.gov/oppsrrd1/REDs/endosulfan_red.pdf.
Van Woerden, H. F. (1963). Organic sulfites. *Chemical Reviews, 63*, 557–571.
Venkatraman, L. (2004). Will banning endosulfan solve the problem? The Hindu business line. July 6. Online at http://www.thehindubusinessline.com/2004/07/06/stories/2004070600790300.htm

Vig, K., Singh, D. K., Agarwal, H. C., Dhawan, A. K., & Dureja, P. (2008). Soil microorganisms in cotton fields sequentially treated with insecticides. *Ecotoxicology Environmental Safety, 69* (2), 263–276.

Weir, K. M., Sutherland, T. D., et al. (2006). A single monooxygenase, ese, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an *Arthrobacter* sp. *Applied and Environment Microbiology, 72*, 3524–3530.

Younglai, E. V., Foster, W. G., Hughes, E. G., & Trimk, J. J. F. (2002). Levels of environmental contaminants in human folicular fluid, serum and seminal uple undergoing invitro fertilization. *Archives of Environmental Contamination and Toxicology, 43*, 121–126.

Yuquan, L. (2000). Genotoxic effects of alpha- endosulfan and beta-endosulfan on human HepG2 cells. *Environmental Health Perspectives, 108*(6), 559–561.

Fungal Degradation of Organochlorine Pesticides

Héctor Hugo León-Santiesteban and Refugio Rodríguez-Vázquez

1 Introduction

The last 20th century brought many scientific and technological advances to human beings. The synthesis of chemical pesticides, capable to prevent, control and eliminate some relevant pests is one of them. The infestation of crop fields with pests has repercussions directly on the quality of foodstuffs and also on economic gains. Therefore, it was imperative to eradicate them. It was until the 1940s, when 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane(DDT) was re-discovered by Müller, which changed scenario by killing the pests in the agricultural fields. Pesticides have saved many economic losses in terms of crop production. Besides, they have prevented the propagation of vector borne diseases, such as: malaria, dengue, yellow fiber, trypanosomias is and typhus in humans. Indeed, the green revolution of many industrialized and non-industrialized nations would have not occurred without their use in agricultural fields. However, overuse of a lot of organochlorinated pesticides during last century has caused serious environmental problems, which have affected human health to a great extent. Besides, it has deteriorated the quality of life of innumerable living organisms, located at hundreds or thousands kilometers far away from the contaminated sites.

A pesticide can be considered as a substance or mixture of substances able to prevent, destroy, repel or mitigate any pest (EPA 2015a). They are classified depending on their use, origin (chemical or biological) and/or chemical nature. The most common way to classify them is by use of pest to be eliminated. For instance: algaecides are used to kill or control of algae; antimicrobials and disinfectants are commonly applied to control bacteria and viruses; fungicides are used to control fungal contaminations; herbicides are applied to kill or inhibit the growth of weeds

H.H. León-Santiesteban · R. Rodríguez-Vázquez (✉)
Departamento de Biotecnología Y Bioingeniería, Centro de Investigación Y de Estudios Avanzados Del Instituto Politécnico Nacional, Mexico City, Mexico
e-mail: rrodrig@cinvestav.mx

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_6

and insecticides to kill insects. Growth regulators are necessary to control and disrupt the growth and reproduction of insects; rodenticide is used to kill mice, rats and gophers; and wood preservatives are employed to make wood resistant to divers pests (EPA 2015b). Although pesticide classification is not clear based on its use, hence it is limited to information about mechanisms of action and chemical nature of pesticides. However, the chemical classification is more useful, for the research point of view. Now pesticides are grouped according to both chemical structure and functional groups. This classification divides them into four main clusters, namely organochlorines, organophosphates, carbamates and pyrethroids (Pereira 2014).

Organochlorine pesticides (OCPs) are a diverse group of organic molecules, that are characterized by having five or more chlorine atoms in their structures (Zacharia 2011). These compounds can be organized into three sub-groups, namely (1) isomers, (2) cyclodienes, and (3) dichlorodiphenyl-ethanes (DDT and analogues) (Maroni et al. 2000). In spite of their structural variations, OCPs share some physico-chemical properties. They are molecules with low solubility in water and high solubility in organic solvents. Hence, they are accumulated in organic matter in soils, aquatic sediments and lipids from living organisms. Environmental agents, such as oxygen, water and light, do not alter their chemical structures, and unfortunately, their biotransformation by micro- and upper organisms is fairly limited (Mrema et al. 2013). Further, OCPs are semi-volatile compounds; hence they can be volatilized to the atmosphere. Therefore, OCPs (as gases or aerosols) are transported by airstream to other places, where they are condensed due to decreasing atmospheric temperature (cold condensation) and finally fall down in condensation phase to water, soils and biota, generating a new contamination site. OCPs from a new contamination site can be re-volatilized to the atmosphere and re-starting the migration cycle under seasonal temperature changes. This atmospheric phenomena, so-called global distillation or fractionation, is responsible for dispersing OCPs from warm places (tropical and subtropical regions) to both high altitude and cold places (mainly mountainous zones, Arctic and Antarctic regions), where low temperatures, little biological activity, lack of soil leaching, few penetration of sunlight and lack of snow break are the main factors for concentrating them into cold environments (Wania and Mackay 1993, 1996; Jones and de Voogt 1999; Fernández and Grimalt 2003; Kelly et al. 2007).

Nowadays, OCPs production and application are banned in many industrialized countries, however, their applications are still allowed in some special cases. DDT, for instance, is currently applied to various developing countries to eradicate malaria vectors. Due to their hardly-degradable chemical structure, physicochemical properties, global distribution, toxic effects on biota and trend to build-up in organic matter and fatty tissues of living organisms, nine of the most over-exploited OCPs worldwide (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene) as well as other organic compounds, like dioxins, furans and polychlorinated biphenyls (PCBs)were classified as Persistent Organic Pollutants (POPs)during the Stockholm Convention on POPs held in 2001. In such convention, it was decided that the participating governments would take all necessary measures to reduce or eliminate POPs from stockpiles and wastes, using efficient and environmentally-friendly management. The twelve organic

compounds, called as POPs, were grouped in three categories, namely annex A, B and C. POPs, such as aldrin, dieldrin, endrin, chlordane, heptachlor, hexachloror, hexachlorobenzene, mixed, toxaphene and the dielectric fluids, PCBs, were listed in annex A, which means that their production and use should be eliminated. However, DDT was listed in annex B and its output and use would be only allowed to prevent the proliferation of vector borne diseases, specifically malaria. Besides, unintentionally formed compounds, such as dioxins, furans and PCBs, were listed in annex C, because they are highly toxic for humans and other living organisms. The release of these compounds into environment should be avoided or minimized where ever possible (Vassilev and Kambourova 2006; Stockholm Convention 2015a).

Later, in 2009, other nine organic compounds were named as POPs during the fourth meeting of the conference of the parties to Stockholm Convention. Five of which were OCPs, such asα- and β-hexachlorocyclohexane, chlordecone, lindane and pentachlorobenzene. The remaining new POPs were hexabromobiphenyl, commercial octabromodiphenyl ether, perfluorooctane sulfonic acid and other halo compound (the commercial pentabromodiphenyl ether). All the new POPs were grouped in annex A, with the exception of other halo compound (perfluorooctane sulfonic acid), which was grouped in annex B. However, pentachlorobenzene was also assigned to annex C (Stockholm Convention 2015c).

It was until the fifth and sixth meeting of the conference of the parties of the Stockholm Convention held in 2011 and 2013, respectively, when technical endosulfan and its related isomers and hexabromocyclododecane were included as POPs; both compounds in annex A (Stockholm Convention 2015c). Currently, decabromodiphenyl ether, dicofol, short-chained chlorinated paraffins, chlorinated naphthalenes, hexachlorobutadiene and pentachlorophenol are in the process of evaluation to be classified as POPs. Unfortunately, five of the new candidates are chlorinated organic compounds (Stockholm Convention 2015b).

This chapter deals with the state of art of the fungal degradation of the first nine OCPs classified as POPs.

2 Degradation of Organochlorine Pesticides Belonging to the Dirty Dozen

2.1 DDT and Its Related Compounds

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, also known as DDT, was first synthesized by the Austrian scientist Othmar Zeidler in 1874 and it was re-discovered and applied as insecticide by Paul Hermann Müller, a Swiss chemist, in 1939. In 1944, England and United States began a large-scale industrial production of DDT. Currently, the use of DDT in several developed and developing countries are banned. However, the incessant use of DDT during the second middle of 21th century led to a worldwide pollution which caused detrimental effects on both wildlife and human health.

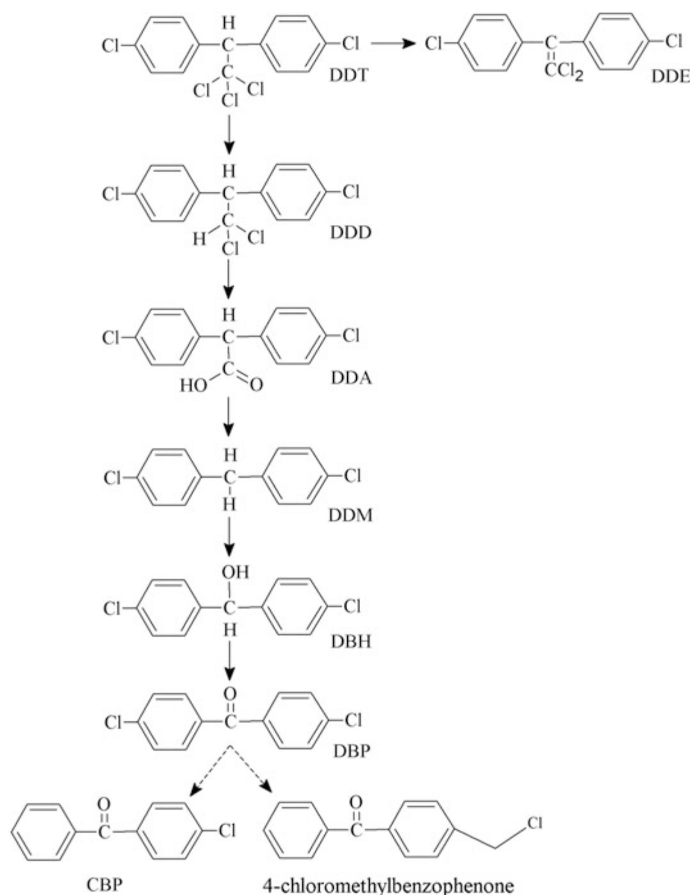


Fig. 1 Fungal degradation of DDT and analogues as proposed by Subba-Rao and Alexander (1985)

Fortunately, a wide range of fungi were found capable to degrade DDT, even some fungal strains mineralized it. Subba-Rao and Alexander (1985) discovered that *Aspergillus flavus* ATCC 11495, *Aspergillus flavus* NRRL 1779, *Thanatephorus cucumeris* and False smut of rice were able to degrade DDT to 1,1-dichloro-2,2-bis (4-chloropheny)ethylene (DDE), 1,1-dichloro-2,2-bis(4-chloropheny)ethane (DDD), 4,4'-dichlorobenzhydrol (DBH) and 4'4-dichlorobenzophenone (DBP), as unique metabolites. On the other hand, they observed that other fungal strains (*Aspergillusniger*, *Apergillus conicus*, *Penicillium brefeldianum*, *Pycnidiophora dispersa*, *Cercospora oryzae*, *Pyricularia oryzae, Schizophyllum* sp. and *Mucor alternans*) were not capable of degrading DDT, but they could transform DDT metabolites to other metabolites. Figure 1 reflects the DDT metabolic pathway as proposed by Subba-Rao and Alexander (1985), which combines the metabolic capabilities of several fungi.

As first degradation reactions, some fungi may degrade DDT by dehydrodechlorination to form DDE and by reductive dechlorination to DDD. Then, the latter is further degraded to bis(4-chlorophenyl)acetic acid (DDA), bis (4-chlorophenyl)methane (DDM), DBH and DBP. Further, *A. niger* was able to degrade DBP to 4-chlorobenzophenone (CBP) and 4-chloromethylbenzophenone.

As to many DDT metabolites from fungal cultures had already been identified in bacterial cultures, Subba-Rao and Alexander (1985) proposed the possibility that the DDT degradation by fungi might be similar to degradation by bacteria. However, Austand and co-workers (Bumpus et al. 1985, 1993; Bumpus and Aust 1987) demonstrated that the DDT degradation by *Phanerochaete chrysosporium* yielded different metabolites from those produced by DDT-degrading bacteria. Nevertheless, it is true that DDT degradation by fungi and bacteria have also some breakdown products in common.

Under nitrogen limitation, *P. chrysosporium* is able to degrade DDT in liquid cultures to by-products and subsequently oxidize them up to carbon dioxide (CO_2). The DDT mineralization by *P. chrysosporium* depended on the type of carbon source (glucose, cellulose, starch, etc.) and its dose (Bumpus et al. 1985; Fernando et al. 1989). Using glucose as carbon source, the DDT mineralization began after third day of incubation. During the first 3 days, when there was no mineralization, subsequently, a reductive dechlorination of DDT to DDD was carried out. Then, DDT and DDD were hydroxylated to dicofol [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol]

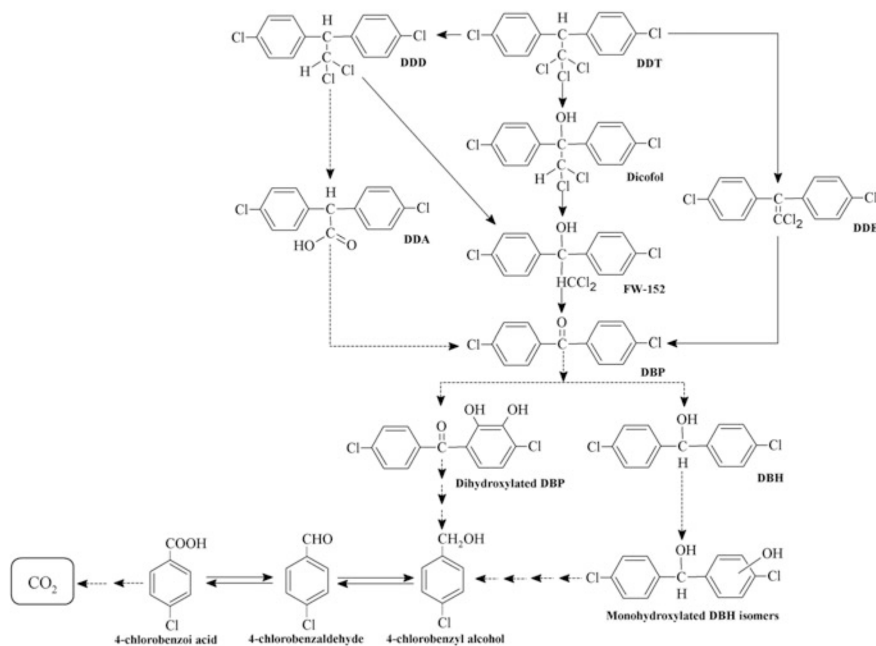


Fig. 2 Pathway for DDT degradation by basidiomycetes (Bumpus et al. 1985, 1993; Bumpus and Aust 1987; Fernando et al. 1989; Xiao et al. 2011c)

and FW-152 [2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol], respectively. After the sixth day, DBP was identified as a metabolite (Fig. 2).

Unfortunately, Bumpus and Aust (1987) failed to identify some key metabolites when they tried to understand the synthesis of DBP from FW-152, which might involve reactions of reductive dechlorination, oxidation and decarboxylation, and the ring cleavage products from DBP transformation. Fernando et al. (1989) identified DDE as breakdown product of DDT, when *P. chrysosporium* was cultured in silt loam soil amended with corn cobs and DDT. Meanwhile, Bumpus et al. (1993) found that during nitrogen starvation, *P. chrysosporium* was able to mineralize DDE with the formation of three metabolites, one of which was DBP (Fig. 2). Aust (1990) proved that the lignin-degrading enzymes from *P. chrysosporium* were not completely involved in the DDT mineralization. It was evident from the fact that when lignin peroxidase activity was inhibited by tetramethylenediamine, the mineralization was decreased, but not inhibited completely.

Zheng et al. (2012) observed that when the solubility of DDT in an aqueous phase was increased by Tween 80 microemulsions, the DDT degradation by *P. chrysosporium* ACCC 30553 was increased, too. In fact, microemulsions enhanced the transport of DDT (dissolved and non-dissolved) to fungal mycelium, promoting the biosorption of DDT. In 28-day cultures with 1000 mg L^{-1} of Tween 80 microemulsions, *P. chrysosporium* ACCC 30553 degraded 74.2 % of 25 ppm DDT and produced DDD, DDE and DBP as metabolites.

Recently, Xiao et al. (2011c) discovered that *Phlebia lindtneri* and *Phlebia brevispora*, in nitrogen-deficient liquid medium, were capable to degrade DDT to DBP through formation of DDD and DDA. There was no formation of dicofol and FW-152. Then, DBP was hydroxylated to dihydroxylated DBP and also reduced to 4,4-dichlorobenzhydrol (DBH). The latter was subsequently hydrolyzed to two monohydroxylated DBH isomers in *P. lindtneri* cultures. Further, 4-chlorobenzal dehyde, 4-chlorobenzyl alcohol and 4-chlorobenzoic acid were identified as single-ring aromatic metabolites, which might be formed by the aromatic ring cleavage of hydroxy-DBP/DBH (Fig. 2). Xiao et al. (2011c) concluded that in the *Phlebia* species, the intracellular Cytochrome P-450 enzymes, were partly responsible for catalyzing hydroxylation of DBP/DBH and ring cleavage reaction of the hydroxylated metabolites of DBP. When these enzymes were inhibited, DBP was accumulated and the synthesis of single-ring aromatic metabolites decreased considerably. However, Cytochrome P-450 enzymes were not found responsible for degrading DDT to DBP.

Fan et al. (2013) carried out the degradation of commercial grade DDT (a blend of *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDT) in soil using a co-remediation process, in which soil was amended with two biocatalysts; a fungus and a purified enzyme. They removed 66.82 % of the initial DDT concentration (4.272 mg kg^{-1}) in 28 days, using 5 ml of fungal inoculum of *Flammulina velutipes* per 15 g soil and 6 U laccase per each gram of soil. The 2,2-bis(4-chlorophenyl)ethanol (DDOH) and p,p'-2,2-bis(chlorophenyl)-1-chloroethane (DDMS) were identified as the breakdown products of commercial grade DDT. However, it is possible that

p,p'-DDE was present in the DDT mixture from the very beginning and also as a metabolite, therefore, its initial concentration was increased and then decreased gradually.

Kulshrestha and Kumari (2010) also found DBP as metabolite during the degradation of DDT by *Fusarium* sp. strain GFSM-4 (ITCC 6841), a fungus isolated from sewage sludge. This fungus was capable to degrade up to 94 % of 50 ppm DDT in 3 weeks in nitrogen-deficient liquid cultures, using mannitol as a carbon source, and 26.94 % of 50 ppm DDT in soil.

On the other hand, Izcapa-Treviño et al. (2009) proposed degradation of DDT and its analogues organochloride compounds by *Penicillium* sp. through a Fenton reaction (H_2O_2/Fe). The peroxide and the free radicals were detected in the culture broth of *Penicillium* sp., amended with $FeSO_4$ under acidic conditions. Thus 10 ppm of commercial DDT was degraded to 92.8 % in 48 h incubation.

2.2 Cyclodiene Pesticides (Aldrin, Dieldrin, Endrin, Chlordane and Heptachlor)

2.2.1 Aldrin and Dieldrin

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimet hanonaphthalene) was synthesized in 1948 and was begun to applied in soils to eradicate insects, such as: termites, grasshoppers, corn rootworm, etc. In the 1970s, use of aldrin was banned in many developed countries. However, it is estimated that between 1946 and 1976, approximately 270 thousands of tons of aldrin and other cyclodiene pesticides were produced (Zitko 2003; Mrema et al. 2013).

In fact, aldrin is not itself toxic to insects or even humans. But, it can be metabolically activated through epoxidation reactions to dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene), which is the true pesticide (Zitko 2003; Mrema et al. 2013).

P. chrysosporium, in nitrogen starvation, has been able to both epoxidizealdrin to dieldrin and degrade aldrin to more polar metabolites. Till date, these metabolites have not been identified (Kennedy et al. 1990). Xiao et al. (2011a) also found dieldrin as product of epoxidation of aldrin during the cultures of *Phlebia acanthocystis*, *Phlebia brevispora* and *Phlebia aurea*. Indeed, white rot fungi, under nitrogen limitation, were able to degrade aldrin and dieldrin to their corresponding metabolites.

P. acanthocystis, *P. brevispora* and *P. aurea* removed 96, 97.6 and 96.4 % of 25 μM aldrin in 42 days, forming 10, 9 and 8.5 μM dieldrin, respectively. The fungal degradation of aldrin yielded 9-hydroxyaldrin, dihydrochlordenedicarboxylic acid and monohydroxydihydrochlordenedicarboxylic acid as by-products, which suggests that these fungi could degrade aldrin through both hydroxylation

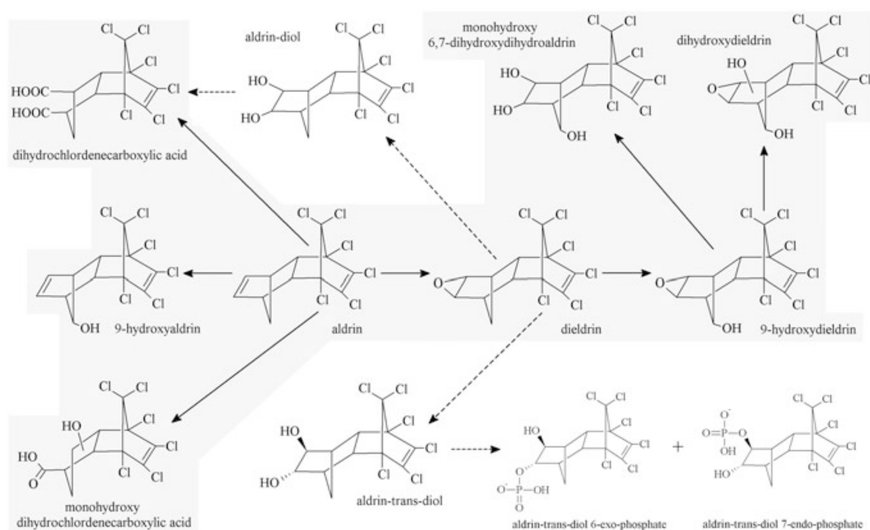


Fig. 3 Fungal metabolic pathways for the degradation of both aldrin and dieldrin. The breakdown products in grey were identified in cultures of *Phlebia* strains (Xiao et al. 2011a). The remaining metabolites were identified in *Mucor racemosus* DDF cultures (Kataoka et al. 2010; Yamazaki et al. 2014)

reaction at methylene moiety and oxidation reactions at non-chlorinated portion of aldrin molecule (Fig. 3).

As far as dieldrin is concerned *P. acanthocystis*, *P. brevispora* and *P. aurea* removed an average of 50 % of the initial concentration of dieldrin (25 μM) in 42 days. In the three fungal cultures, 9-hydroxydieldrin was identified as the key metabolite. Nevertheless, the hydroxylation at nine position of dieldrin by a strain of *P. brevispora*, was first observed by Kamei et al. (2010). In cultures of *Phlebia* strains, 9-hydroxydieldrin was subsequently metabolized to both dihydroxydieldrin and monohydroxy 6,7-dihydroxydihydroaldrin through hydroxylation reactions at non-chlorinated site and at the epoxy ring to produce a diol compound(Fig. 3).

On the other hand, *Mucor racemosus* strain DDF was capable to carry out the epoxide ring-opening of dieldrin via hydroxylation, which led to the formation of aldrin-*trans*-diol (6,7-trans-dihydroxydihydroaldrin) as by-product. This hydroxylation was possibly catalyzed by an extracellular epoxide hydrolase. The spent culture broth, by itself, was able to degrade 46 % of 13.2 μM dieldrin, while the whole cultures (broth and biomass) degraded 95.8 % of dieldrin in approximately 21 days (Kataoka et al. 2010). Later, Yamazaki et al. (2014) discovered that the second phase of dieldrin degradation, namely the degradation of aldrin *trans*-diol, by *Mucor racemosus* strain DDF was through conjugation reactions with phosphate and sulfate groups. Aldrin-*trans*-diol 6-exo-phospahte,aldrin-*trans* 7-endo-phospahte and a diastereomeric sulfate of aldrin-*trans*-diol were identified as conjugated metabolites of aldrin-*trans*-diol (Fig. 3).

Xiao and Kondo (2013a) used fungi belonging to genus *Cordyceps* (*C. brongniartii* ATCC66779 and *C. militaris* KS-92) for degrading dieldrin in liquid cultures and discovered 6, 7-dihydroxydihydroaldrin as a main metabolite. However, *C. militaris* KS-92 was able to degrade 6,7-dihydroxydihydroaldrin to dihydrochlordenedicarboxylic acid, which was alone noted as a breakdown product of aldrin by *Phlebia* strains.

Although several fungi have been able to degrade dieldrin, but only a few dieldrin-mineralizing fungi have been reported in the literature. Only *Trichoderma koningi*, *P. chrysosporium* ATCC 24725, *Chrysosporium lignorum* and *Trametes versicolor* have been reported to achieve mineralization of dieldrin (Bixby et al. 1971; Morgan et al. 1991).

2.2.2 Endrin

Endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *endo*-5,8-dimethanonaphthalene) is the *endo, endo* stereoisomer of dieldrin and it was mainly used to control the insect pests of cotton. Although, it was also applied to prevent the proliferation of pests on rice, sugar cane, grain crops, sugar beets, maize, tobacco and cole crops. Sporadically, it was used as a rodenticide and as a treatment for cotton and beans seeds. As a particular and unfavorable attribute, endrin is rather more toxic to non-target organisms than aldrin and dieldrin (Zitko 2003; Mrema et al. 2013).

Since 1960s until 2010s, the vast majority of the biodegradation studies of endrin have been performed with bacteria, hence the fungal degradation of endrin has been little known. Patil et al. (1970) observed that *Tricoderma viride* (strain 12 and 41) were capable to metabolize endrin yielding metabolites; one of which was identified as keto-endrin (Fig. 4). This was also identified during the degradation of endrin with a yeast (unidentified) isolated from a farm soil by Matsumura et al. (1971).

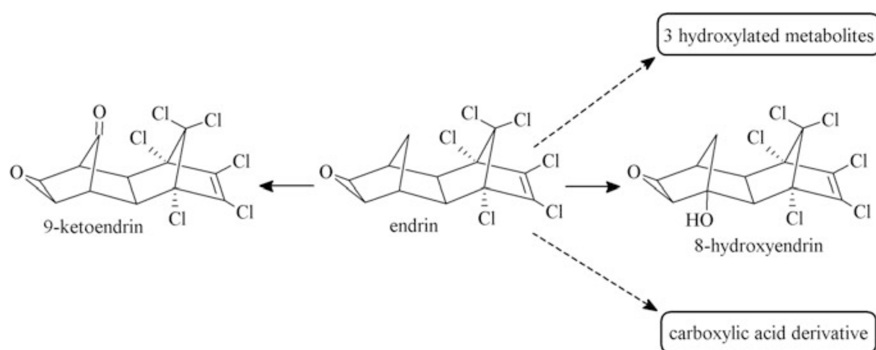


Fig. 4 Endrin metabolites yielded by fungal metabolism as identified by Patil et al. (1970), Matsumura et al. (1971) and Xiao and Kondo (2013b)

Recently, Xiao and Kondo (2013b) discovered that the fungus *Phlebia acanthocystis* TMIC34875 could degrade endrin through hydroxylation and oxidation reactions. In the liquid cultures of *P. acanthocystis* TMIC34875, at least five metabolites were detected. Three were classified as hydroxylated derivatives of endrin; one of which was identified as 8-hydroxyendrin (Fig. 4). Further, a carboxylic acid derivative of endrin was partially identified.

2.2.3 Chlordane and Heptachlor

In 1948, the technical chlordane was introduced to the market of pesticides, as an agent against insects and undesirable plants. After 1979, the principal use of technical chlordane was against termites in the United States. It was until 1988 when the sales and application of technical chlordane were voluntarily stopped (Dearth and Hites 1991).

Technical grade chlordane was composed by more than 120 chlorinated compounds. Later on 147 components were detected. In the oldest technical mixture, the most abundant components were the chlordane isomers (α or *cis* and γ or *trans*; less than 60 %) and heptachlor (up to 10 %). Over the years, the formulation of technical chlordane changed to 70 % of the *cis*-chlordane, 25 % of the *trans*-chlordane and less than 1 % of heptachlor (Tafuri et al. 1977; Dearth and Hites 1991; Mattina et al. 1999).

The ascomycete *Aspergillus niger*, in aqueous medium, could remove between 87.5 and 100 % of 12.5 and 50 ppm chlordane in 48 h, but it was incapable of forming breakdown products (Iyengar and Rao 1973). On the contrary, the basidiomycete *P. chrysosporium* BKM-F-1767 could degrade chlordane up to CO_2 in both submerged and soil-corncob cultures in 30 days. However, the metabolites of chlordane were not characterized (Kennedy et al. 1990).

On the other hand, Xiao et al. (2011d) showed that some representative strains of the genus *Phlebia* were able to transform *trans*-chlordane to other toxic and/or recalcitrant cyclodienes. Solely heptachlor, heptachlor epoxide, oxychlordane and dichlorochlordene were fully identified as transformation products of *trans*-chlordane. However, chlordane chlorohydrin, monohydroxychlordene, 3-hydroxyxhlordane, a hydroxylatedderivative of oxychlordane, heptachlordiol, dihydroxydihydrochlordene and dihydroxychlordene were partially identified as metabolites, because their molecules were structurally deduced by mass spectrometry. Figure 5 depicts the fungal transformation of *trans*-chlordane as suggested by Xiao et al. (2011d).

When the intracellular Cytochrome P450 enzymes from *P. lindtneri* were inhibited, some steps of chlordane metabolism were stopped, particularly related to the epoxidation and hydroxylation reactions. Afterwards, Xiao and Kondo (2013c) determined that both extra- and intracellular enzymes produced by *P. brevispora* had the catalytic potential for transforming chlordane. The extracellular enzymatic extract was responsible to yield heptachlor, dichlorochlordene, oxychlordane and heptachlor epoxide. Meanwhile, the intracellular enzymatic extract generated

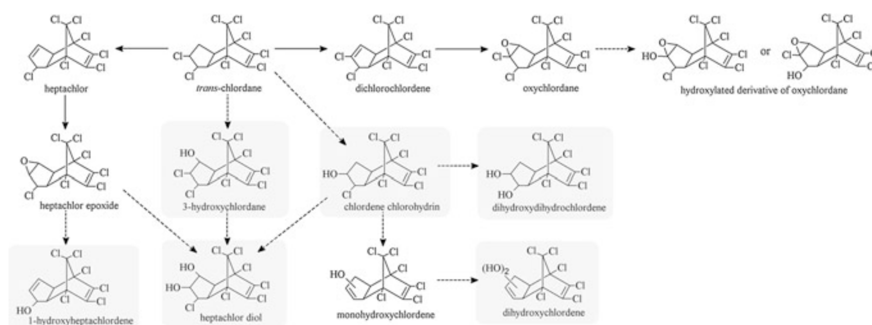


Fig. 5 *Trans*-chlordane degradation pathway in fungi of genus *Phlebia* (Xiao et al. 2011d; Xiao and Kondo 2013c). The metabolites in grey were intracellularly produced

chlordane chlorohydrin, hydroxychlordane, dihydroxydihydrochlordene, 3-hydroxychlordane, heptachlor diol and dihydroxychlordene. In general, the degradation capability of the intracellular enzymes was 2 or 3 times higher than those extracellular enzymes.

Like chlordane, heptachlor was also used as insecticide, mainly against termites. In 1970s, some developed countries banned and limited its production, as it was found that heptachlor is a probable carcinogen in humans. Although, according to Stockholm Convention, heptachlor can be currently used in specific applications, such as: termiticide and as an additive in the underground cable boxes.

Although, heptachlor and chlordane are structurally similar molecules, but heptachlor is not chlorinated at C-2 and has a double bond between C-2 and C-3, while chlordane is chlorinated at C-2 and had a single bond between C-2 and C-3 (Kennedy et al. 1990). Toxicological tests have showed that heptachlor is about 3–5 times more potent as pesticide and more recalcitrant than chlordane (Mrema et al. 2013).

Due to its low water solubility, heptachlor has been successfully removed from an aqueous medium by *A. niger*, *Lentinus subnudus* and *P. chrysosporium*. The latter has converted heptachlor to unidentified by-products in both liquid and soil-corncob cultures (Iyengar and Rao 1973; Kennedy et al. 1990; Nwachukwu and Osuji 2007). Miles et al. (1969) [during their research with soil microorganisms] discovered several fungi of the genus *Trichoderma*, *Penicillium*, *Fusarium* and *Rhizopus* were capable of oxidizing heptachlor to heptachlor epoxide, which is, in fact, a more toxic pesticide than its parent compound. Additionally, 1-hydroxychlordene and 1-hydroxy-2,3-epoxychlordane were identified as metabolites of heptachlor. However, it was observed that 1-hydroxychlordene was produced by non-metabolic reactions (Fig. 6).

Xiao et al. (2011b) found heptachlor epoxide as a main metabolite during the degradation of heptachlor with eighteen fungi belonging to the genus *Phlebia*, in nitrogen-limiting liquid cultures. *P. tremellosa*, *P. brevispora* and *P. acanthocystis* could epoxidize 68, 64 and 75 % of 0.25 μmol heptachlor in 14 days. Further,

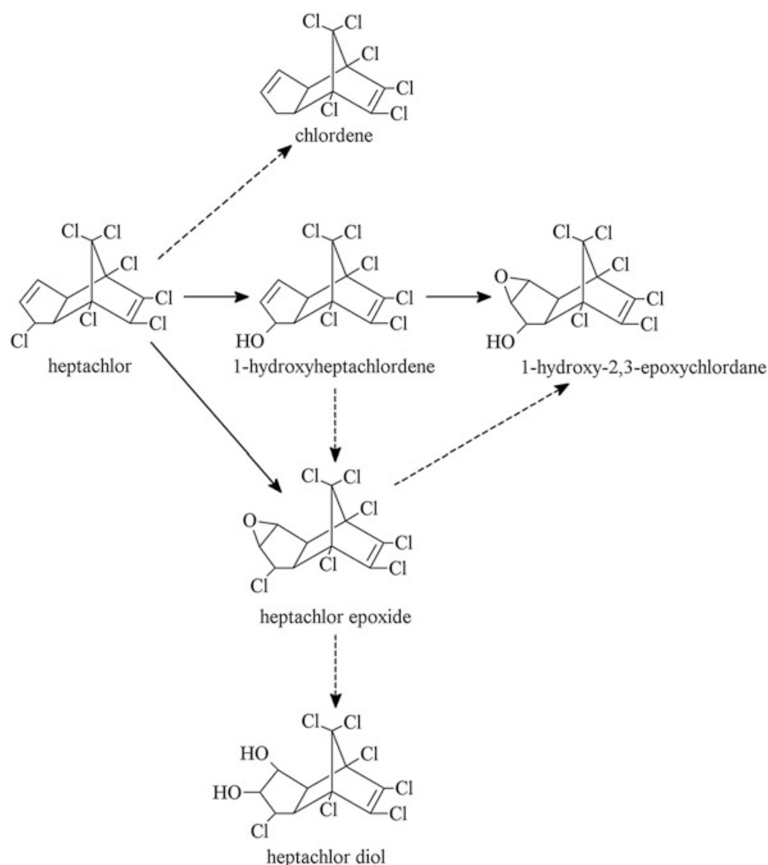


Fig. 6 Fungal degradation of heptachlor. Pathway deduced from the work of Miles et al. (1969), Xiao et al. (2011b) and Purnomo et al. (2013)

heptachlor was also hydrolyzed to 1-hydroxychlordene which was subsequently oxidized to 1-hydroxy-2,3-epoxychlordane (Fig. 6). In these experiments, 1-hydroxychlordene was exclusively formed by metabolic reactions.

When heptachlor epoxide was used as a substrate, *P. aurea*, *P. brevispora* and *P. acanthocystis* were able to yield traces of 1-hydroxy-2,3-epoxychlordane and heptachlor diol (2,3-dihydroxyheptachlor). This means that heptachlor epoxide may be degraded by both oxidative dechlorination (hydroxylation) at 1 position and hydrolysis at the epoxide ring. *P. lindtneri* formed traces of heptachlor diol, but not 1-hydroxy-2,3-epoxychlordane (Fig. 6).

Heptachlor epoxide was also identified as key metabolite of heptachlor in liquid cultures of *Pleurotus ostreatus*. Nonetheless, its formation was not related to the ligninolytic enzymes, due to the fungus epoxidized heptachlor under either nitrogen-limiting or non-limiting conditions, as well as in nutrient-rich medium.

In addition to heptachlor epoxide, 1-hydroxychlordene was yielded by *P. ostreatus* in nitrogen starvation. In high nitrogen and rich-nutrient cultures, the second most abundant metabolite was chlordane followed by 1-hydroxychlordene. In rich-nutrient medium, *P. ostreatus* was able to hydroxidize heptachlor epoxide to heptachlor diol (Purnomo et al. 2013).

Purnomo et al. (2014) observed that the residual solids generated during the mushroom production, known as spent mushroom wastes (SMW), have a very high potential for the degradation of heptachlor and heptachlor epoxide, as the compost material is enriched in nutrients, enzymes, microorganisms and fungal debris. SMW of *P. ostreatus* produced chlordene as the main metabolite of heptachlor in addition to heptachlor epoxide, 1-hydroxychlordene and heptachlor diol. The latter was solely produced as a metabolite of heptachlor epoxide. Apparently, the reductive dechlorination of heptachlor is the predominant process during the degradation of heptachlor rather than epoxidation by SMW of *P. ostreatus*. In contrast, epoxidation of heptachlor was the main process during the heptachlor degradation with SMW of *Phlebia* strains.

2.2.4 Mirex

In 1946, mirex was first synthesized by Prins, although it was only after 1959 when its merchandising began under the name GC-1283; a formulation produced by the Allied Chemical Corporation for killing the imported fire ants. EPA has estimated that approximately 275,000 kg of mirex was applied for controlling the imported fire ants in U.S. between 1959 and 1977. Alternatively, mirex (commercially known as dechloranes) was used as a flame retardant for plastic and polymers. It was been calculated that the amount of mirex, sold as a flame retardant, was three to four times higher than the amount sold as a pesticide. In 1978, the production of mirex was banned by the government of U.S. (Waters et al. 1977; Bell et al. 1979; Huckins et al. 1982).

Mirex is considered as one of the most stable organochlorine pesticide, which has been spilled into environment, and it is extremely resistance to chemical, biochemical, thermal and microbial degradation. Hence, it tends to accumulate into non-living matter as well as biota. Microorganisms from anaerobic sludge have been able to degrade mirex up to a 10-monohydroderivativethrough reductive dechlorination (Fig. 7) (Andrade and Wheeler 1974; Andrade et al. 1975). On the other hand, the white-rot fungus *P. chrysosporium* BKM-F-1767 has shown solely the metabolic capability for mirex degradation. In 30-day soil-corncob cultures, the fungus could mineralize 4 % of 5.2 nmol ^{14}C-mirex and formed an unidentified metabolite, which presence was 9.6 %. On the contrary, in 30-day liquid cultures, *P. chrysosporium* BKM-F-1767 only formed an unidentified metabolite, which presence was equivalent to the 3.6 % of initial ^{14}C-mirex (Fig. 7) (Kennedy et al. 1990).

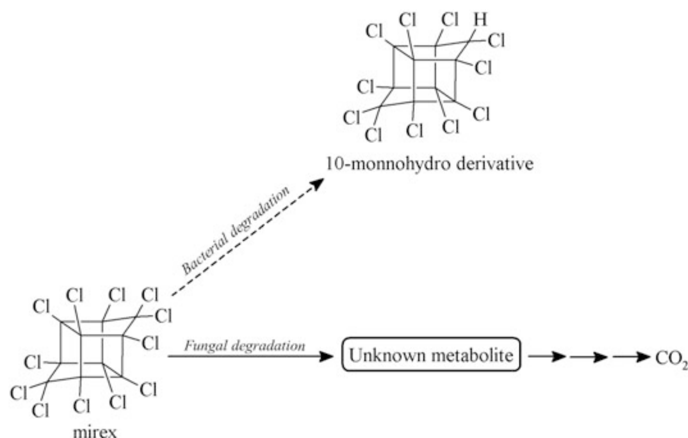


Fig. 7 Degradation of mirex by microorganisms

2.2.5 Toxaphene

The organochlorine pesticide, known as toxaphene, is a complex mixture of hundred polychlorinated compounds, mostly champenes and bornanes, which possess six to nine chlorine atoms. This mixture was applied specifically to eradicate insect pests of cotton, tobacco, forests, turf, ornamental plants, grains, vegetable and livestock. However, it was also used against unwanted fish in lakes and ponds in U.S. In 1982, the license to sell and distribute toxaphene was cancelled by EPA, but it was until 1990 when all its applications were wholly banned in U.S. (Baughman et al. 2005).

Due to its chemical nature, toxaphene has a low water solubility (3 mg L^{-1} at 22 °C), and gets accumulated in either organic or inorganic matter. Paris et al. (1977) observed that *Aspergillus* sp. wet biomass had the capability to sorb toxaphene, but not to degrade it. Indeed, only two fungi have been able to degrade toxaphene. Matsumura and Katayama (1994) patented a system in which some halogenated organic compounds, including toxaphene, can be degraded in liquid medium by both immobilized biomass of *P. chrysosporium* ATCC 64046 and UV light. The patent does not present results about the degradation of toxaphene in liquid medium, but indicated that 52 % of 1 mg toxaphene was degraded in 4 weeks in solid cultures of *P. chrysosporium* irradiated with UV light.

On the other hand, Romero et al. (2006) proved that the white rot fungus *Bjerkandera* sp. strain BOL13 cultured in liquid medium amended with agro-industrial wastes could degrade sundry toxaphene isomers, namely 2,2,3-exo,8,9,10-hexachlorocamphene (P11); 2-exo,3-endo,8,8,9,10-hexachlorocamphene (P12); 2-exo,3-endo,7,8,9,10-hexachlorocamphene (P15); 2,2,3-exo,8,8,10,10-octachlorocamphene (P31); 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (P32); 2,2,3-exo,5-endo,6-exo,8,9,10-octachlorobornane (P39); 2,endo,3-exo,5-endo,6-exo,

8,9,10,10-octachlorobornane (P40); 2,exo,3-endo,5-exo,6-exo,8,9,9,10,10-octachlorobornane (P41); 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane and 2,2,5-endo, 6-exo,8,9,9,10-octachlorobornane (P42); 2-exo,5,5,8,9,9,10-octachlorobornane (P44); 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (P50); and 2,2,5, 5,6-exo,8,9,9,10,10-decachlorobornane (P69). In 38-day cultures with wheat husk as a co-substrate, *Bjerkandera* sp. strain BOL13 degraded 85 % of the total toxaphene (20 mg L^{-1}) and from 70 to 85 % of the toxaphene isomers, except for P32, of which only 53.6 % was degraded. Apparently, wheat husk offers adequate nutrients to *Bjerkandera* sp. strain BOL13, since this fungus could degrade more easily the most chlorinated toxaphene isomers than those with a few chlorine atoms. Further, this fungus, in presence of wheat husk, formed at least 3 unidentified metabolites of toxaphene. Moreover, cultures with cane molasses and wood chips as co-substrates were not efficient for degrading toxapheneas compared to wheat husk. About 49 and 52 % of the total toxaphene and from 43 to 77 % of the toxaphene isomers were degraded by *Bjerkandera* sp. strain BOL13, respectively.

2.2.6 Hexachlorobenzene

The anthropogenic chlorinated aromatic compound, known as hexachlorobenzene (HCB), has been an environmental problem since 1933, when it was first applied as a fungicide on the seeds of onion and sorghum, as well as on the crops of wheat, barley, oats and rye. Industrially, this pesticide has been used during the production of graphite anodes, nitroso and styrene rubber for tyres, pyrotechnics, tracer bullets, aluminum and dyes. Besides, it served as a wood-preserving agent. Unfortunately, this xenobiotic can be also synthesized unintentionally during the manufacture of perchlorethylene, chlorine, carbon tetrachloride, chlorinated solvents and some pesticides. It has been estimated that the maximum production of HCB was achieved from 1978 to 1981, and it was about 10,000 tons per year. Until 2005, the use of HCB had been fully banned in 35 countries. Among which, 8 countries discontinued it and5 countries had restricted its applications (Courtney 1979; Barber et al. 2005).

As it was expected, HCB caused a toxic effect on the growth of some fungal strains. Ruckdeschel and Renner (1986) determined that less than 2 μmol HCB mL^{-1} was sufficient to inhibit completely the growth of the following strains: *Saccharomices cerevisiae* ATCC 18824, *Torulopsis glabrata* DSM 70614, *Candida albicans* ATCC 10321, *C. krusei* DSM 70075, *Geotrichum candidadum* PC 67, *A. fumigates* PC 8, *A. niger* PC 6, *Mucor circinelloides* PC 1, *Absidia glauca* PC 3a, *Rhizopus nigricans* PC2, *Penicillium claviforme* PC 10c, *Scopulariopsis brevicaulis* PC 15 a, *Cephaloascus fragrans* DSM 1942, *Cladosporium herbarum* PC 21, *Stemphylium sarciniforme* DSM 53045 and *Microsporum canis* PC 41.

On the other hand, Matheus et al. (2000) found that nineteen Brazilian fungi were highly tolerant to HCB, when they were cultured into packed soil columns. Two strains could tolerate up to 51,166 mg HCB kg^{-1}, nine strains colonized soil with up to 25,000 mg HCB kg^{-1} and eight strains grew in the presence of

5000 mg HCB kg^{-1}. In 65-day soil cultures amended with wheat grains, $CaSO_4$, sugar cane bagasse and 29,180 mg HCB kg^{-1}, *Psilocybe* cf. *castanella* CCB44 removed on average 10.8 % of HCB and yielded 30.03 mg inorganic Cl^- kg^{-1}. *Trametes villosa* CCB176 cultures where the HCB removal was not statistically different from controls, but 67 mg Cl^- kg^{-1} were produced. Regardless of dechlorination, *P. castanella* CCB44 and *T. villosa* CCB176 were capable of mineralizing less than 1 % of HCB (3.61×10^6 d.p.m of HCB-UL-[^{14}C]100 g^{-1} of soil) in 128-day cultures. The same level of HCB mineralization was also calculated by Vitali et al. (2006), but in 56-day amended soils inoculated with fungi belonging to the genera *Eupenicillium*. Additionally, these fungi formed HCB metabolites which were strongly linked to the soil organic matter, resulting into less bioactive chlorinated compounds.

References

Andrade, P, Jr., Wheeler, W. B., & Carlson, D. (1975). Identification of a Mirex metabolite. *Bulletin of Environment Contamination and Toxicology, 14*(4), 473–479.

Andrade, P. S. L, Jr., & Wheeler, W. B. (1974). Biodegradation of Mirex by sewage sludge organisms. *Bulletin of Environment Contamination and Toxicology, 11*(5), 415–416.

Aust, S. (1990). Degradation of environmental pollutants by *Phanerochaete chrysosporium*. *Microbial Ecology, 20*(1), 197–209.

Barber, J. L., Sweetman, A. J., van Wijk, D., & Jones, K. C. (2005). Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *Science of the Total Environment, 349*(1–3), 1–44.

Baughman, C., Bhattacharyya, T., Schanamann, S., Tafer F. E., & Wilson, M. H. (2005). *Ombudsman report, more information is needed on toxaphene degradation products*. U.S. Environmental Protection Agency, Report No. 2006-00007.

Bell, M. R., Ewing, R. A., Lutz, G. A., & Alley, E. G. (1979). *Reviews of the environmental effects of pollutants: I. Mirex and Kepone*. Washington, DC: US Environmental Protection Agency. EPA/600/1-78/013.

Bixby, M. W., Boush, G. M., & Matsumura, F. (1971). Degradation of dieldrin to carbon dioxide by a soil fungus *Trichoderma koningi*. *Bulletin of Environment Contamination and Toxicology, 6*(6), 491–494.

Bumpus, J., Tien, M., Wright, D., & Aust, S. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. *Science, 228*(4706), 1434–1436.

Bumpus, J. A., & Aust, S. D. (1987). Biodegradation of DDT [1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. *Applied and Environment Microbiology, 53*(9), 2001–2008.

Bumpus, J. A., Powers, R. H., & Sun, T. (1993). Biodegradation of DDE (1,1-dichloro-2,2-bis (4-chlorophenyl)ethene) by *Phanerochaete chrysosporium*. *Mycological Research, 97*(1), 95–98.

Courtney, K. D. (1979). Hexachlorobenzene (HCB): A review. *Environmental Research, 20*(2), 225–266.

Dearth, M. A., & Hites, R. A. (1991). Complete analysis of technical chlordane using negative ionization mass spectrometry. *Environmental Science and Technology, 25*(2), 245–254.

EPA (2015a) About pesticides. http://www.epa.gov/pesticides/about/. Accessed 31 March 2015

EPA (2015b) Pesticides and consumers. http://www2.epa.gov/safepestcontrol/why-we-use-pesticides. Accessed 31 March 2015

Fan, B., Zhao, Y., Mo, G., Ma, W., & Wu, J. (2013). Co-remediation of DDT-contaminated soil using white rot fungi and laccase extract from white rot fungi. *Journal of Soils and Sediments, 13*(7), 1232–1245.

Fernández, P., & Grimalt, J. O. (2003). On the global distribution of persistent organic pollutants. *Chimia, 57*(9), 514–521.

Fernando, T., Aust, S. D., & Bumpus, J. A. (1989). Effects of culture parameters on DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] biodegradation by *Phanerochaete chrysosporium*. *Chemosphere, 19*(8–9), 1387–1398.

Huckins, J. N., Stalling, D. L., Petty, J. D., Buckler, D. R., & Johnson, B. T. (1982). Fate of Kepone and mirex in the aquatic environment. *Journal of Agriculture and Food Chemistry, 30* (6), 1020–1027.

Iyengar, L., & Rao, A. V. S. P. (1973). Metabolism of chlordane and heptachlor by *Aspergillus niger*. *Journal of General and Applied Microbiology, 19*(4), 321–324.

Izcapa-Treviño, C., Loera, O., Tomasini-Campocosio, A., Esparza-García, F., Salazar-Montoya, J.A., Díaz-Cervantes, M.D., et al. (2009). Fenton (H_2O_2/Fe) reaction involved in *Penicillium* sp. culture for DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane)] degradation. *Journal of Environmental Science and Health*, *Part B, 44*(8), 798–804

Jones, K. C., & de Voogt, P. (1999). Persistent organic pollutants (POPs): State of the science. *Environmental Pollution, 100*(1–3), 209–221.

Kamei, I., Takagi, K., & Kondo, R. (2010). Bioconversion of dieldrin by wood-rotting fungi and metabolite detection. *Pest Management Science, 66*(8), 888–891.

Kataoka, R., Takagi, K., Kamei, I., Kiyota, H., & Sato, Y. (2010). Biodegradation of dieldrin by a soil fungus isolated from a soil with annual endosulfan applications. *Environmental Science and Technology, 44*(16), 6343–6349.

Kelly, B. C., Ikonomou, M. G., Blair, J. D., Morin, A. E., & Gobas, F. A. P. C. (2007). Food web-specific biomagnification of persistent organic pollutants. *Science, 317*(5835), 236–239.

Kennedy, D. W., Aust, S. D., & Bumpus, J. A. (1990). Comparative biodegradation of alkyl halide insecticides by the white rot fungus, *Phanerochaete chrysosporium* (BKM-F-1767). *Applied and Environment Microbiology, 56*(8), 2347–2353.

Kulshrestha, G., & Kumari, A. (2010). Simultaneous degradation of mixed insecticides by mixed fungal culture isolated from sewage sludge. *Journal of Agriculture and Food Chemistry, 58* (22), 11852–11856.

Maroni, M., Colosio, C., Ferioli, A., & Fait, A. (2000). Introduction. *Toxicology, 143*(1), 5–118.

Matheus, D., Bononi, V., & Machado, K. (2000). Biodegradation of hexachlorobenzene by basidiomycetes in soil contaminated with industrial residues. *World Journal of Microbiology and Biotechnology, 16*(5), 415–421.

Matsumura, F., & Katayama, A. (1994). Photochemically enhanced microbial degradation of environmental pollutants. *United States Patent, 5*(342), 779.

Matsumura, F., Khanvilkar, V. G., Patil, K. C., & Boush, G. M. (1971). Metabolism of endrin by certain soil microorganisms. *Journal of Agriculture and Food Chemistry, 19*(1), 27–31.

Mattina, M. J. I., Iannucci-Berger, W., Dykas, L., & Pardus, J. (1999). Impact of long-term weathering, mobility, and land use on chlordane residues in soil. *Environmental Science and Technology, 33*(14), 2425–2431.

Miles, J. R. W., Tu, C. M., & Harris, C. R. (1969). Metabolism of heptachlor and its degradation products by soil microorganisms. *Journal of Economic Entomology, 62*(6), 1334–1338. doi:10.1093/jee/62.6.1334

Morgan, P., Lewis, S., & Watkinson, R. (1991). Comparison of abilities of white-rot fungi to mineralize selected xenobiotic compounds. *Applied Microbiology and Biotechnology, 34*(5), 693–696.

Mrema, E., Rubino, F., & Colosio, C. (2013). Obsolete pesticides —a threat to environment, biodiversity and human health. In L. I. Simeonov, F. Z. Macaev & B. G. Simeonova (Eds.),

Environmental security assessment and management of obsolete pesticides in Southeast Europe (pp. 1–21). NATO science for peace and security series C: Environmental security. Springer, Netherlands.
Nwachukwu, E. O., & Osuji, J. O. (2007). Bioremedial degradation of some herbicides by indigenous white rot fungus, *Lentinus subnudus*. *Journal of Plant Sciences, 2*, 619–624.
Paris, D., Lewis, D., & Barnett, J. (1977). Bioconcentration of toxaphene by microorganisms. *Bulletin of Environment Contamination and Toxicology, 17*(5), 564–572.
Patil, K. C., Matsumura, F., & Boush, G. M. (1970). Degradation of endrin, aldrin, and DDT by soil microorganisms. *Applied Microbiology, 19*(5), 879–881.
Pereira, L. (2014). Persistent organic chemicals of emerging environmental concern. In A. Malik, E. Grohmann, & R. Akhtar (Eds.), *Environmental deterioration and human health* (pp. 163–213). Netherlands: Springer.
Purnomo, A., Putra, S., Shimizu, K., & Kondo, R. (2014). Biodegradation of heptachlor and heptachlor epoxide-contaminated soils by white-rot fungal inocula. *Environmental Science and Pollution Research, 21*(19), 11305–11312.
Purnomo, A. S., Mori, T., Putra, S. R., & Kondo, R. (2013). Biotransformation of heptachlor and heptachlor epoxide by white-rot fungus *Pleurotus ostreatus*. *International Biodeterioration and Biodegradation, 82*, 40–44.
Romero, M., Terrazas, E., van Bavel, B., & Mattiasson, B. (2006). Degradation of toxaphene by *Bjerkandera* sp. strain BOL13 using waste biomass as a cosubstrate. *Applied Microbiology and Biotechnology, 71*(4), 549–554.
Ruckdeschel, G., & Renner, G. (1986). Effects of pentachlorophenol and some of its known and possible metabolites on fungi. *Applied and Environment Microbiology, 51*(6), 1370–1372.
Stockholm Convention (2015a) The 12 initial POPs under the Stockholm Convention. http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx. Accessed 31 March 2015
Stockholm_Convention (2015b) Chemicals proposed for listing under the Convention. http://chm.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing/tabid/2510/Default.aspx. Accessed 31 March 2015
Stockholm_Convention (2015c) The new POPs under the Stockholm Convention. http://chm.pops.int/Implementation/NewPOPs/TheNewPOPs/tabid/672/Default.aspx. Accessed 31 March 2015
Subba-Rao, R. V., & Alexander, M. (1985). Bacterial and fungal cometabolism of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and its breakdown products. *Applied and Environment Microbiology, 49*(3), 509–516.
Tafuri, F., Businelli, M., Scarponi, L., & Marucchini, C. (1977). Decline and movement of AG chlordane in soil and its residues in alfalfa. *Journal of Agriculture and Food Chemistry, 25*(2), 353–356.
Vassilev, K., & Kambourova, V. (2006). Pesticides as global environmental pollutants. In L. Simeonov & E. Chirila (Eds.), *Chemicals as intentional and accidental global environmental threats* (pp. 173–191)., NATO security through science series Netherlands: Springer.
Vitali, V. M. V., Machado, K. M. G., Andrea, M. M. D., & Bononi, V. L. R. (2006). Screening mitosporic fungi for organochlorides degradation. *Brazilian Journal of Microbiology, 37*, 256–261.
Wania, F., & Mackay, D. (1993). Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. *Ambio, 22*(1), 10–18.
Wania, F., & MacKay, D. (1996). Peer reviewed: Tracking the distribution of persistent organic pollutants. *Environmental Science and Technology, 30*(9), 390A–396A.
Waters, E. M., Huff, J. E., & Gerstner, H. B. (1977). Mirex. An overview. *Environmental Research, 14*(2), 212–222.
Xiao, P., & Kondo, R. (2013a). Biodegradation of dieldrin by *Cordyceps* fungi and detection of metabolites. *Applied Mechanics and Materials, 295–298*, 30–34.

Xiao, P., & Kondo, R. (2013b). Degradation characteristics and mechanism of endrin by a white rot fungus, *Phlebia acanthocystis* TMIC34875. *Chinese Journal of Applied and Environmental Biology, 19*(1), 119–125.

Xiao P, Kondo R (2013c) Enzymatic degradation of organochlorine insecticide chlordane by *Phlebia brevispora*. *Agricultural Biotechnology, 2*(4):53–56, 60.

Xiao, P., Mori, T., Kamei, I., Kiyota, H., Takagi, K., & Kondo, R. (2011a). Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus Phlebia. *Chemosphere, 85*(2), 218–224.

Xiao, P., Mori, T., Kamei, I., & Kondo, R. (2011b). Metabolism of organochlorine pesticide heptachlor and its metabolite heptachlor epoxide by white rot fungi, belonging to genus *Phlebia*. *FEMS Microbiology Letters, 314*(2), 140–146.

Xiao, P., Mori, T., Kamei, I., & Kondo, R. (2011c). A novel metabolic pathway for biodegradation of DDT by the white rot fungi, *Phlebia lindtneri* and *Phlebia brevispora*. *Biodegradation, 22* (5), 859–867.

Xiao, P., Mori, T., & Kondo, R. (2011d). Biotransformation of the organochlorine pesticide trans-chlordane by wood-rot fungi. *New Biotechnology, 29*(1), 107–115.

K-i, Yamazaki, Takagi, K., Kataoka, R., Kotake, M., Yamada, T., & Kiyota, H. (2014). Novel phosphorylation of aldrin-trans-diol by dieldrin-degrading fungus *Mucor racemosus* strain DDF. *International Biodeterioration and Biodegradation, 92*, 36–40.

Zacharia JT (2011) Identity, physical and chemical properties of pesticides. *Pesticides in the modern world—trends in pesticides analysis*. doi:10.5772/17513.

Zheng, G., Selvam, A., & Wong, J. W. C. (2012). Oil-in-water microemulsions enhance the biodegradation of DDT by Phanerochaete chrysosporium. *Bioresource Technology, 126*, 397–403.

Zitko, V. (2003). Chlorinated pesticides: aldrin, DDT, endrin, dieldrin, mirex. In H. Fiedler (Ed.), *Persistent organic pollutants* (Vol. 3O, pp. 47–90)., The handbook of environmental chemistry Berlin Heidelberg: Springer.

Microbial Degradation of Endsulfan and Endsulfan Sulfate

Ryuichiro Kondo

1 Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a, 6,9,9a-hexa- hydro-6, −9 methano-2,3,4-benzo-dioxathiepin-3-oxide also known as Thiodan, Cyclodan and Parrysulfon commercially) is a cyclodieneorganochlorine insecticide that possesses a relatively broad spectrum of activity. It has been used worldwide in a variety of crops, such as pear, broccoli, squash, potatoes, cereals, coffee, cotton, and oilseeds for protection against pest infestation (Hatipoglu et al. 2008; Elsaid et al. 2010; Kalyani et al. 2010; Singh and Singh 2011).

Commercial-grade endosulfan is a mixture of two stereoisomers, namely α- and β-endosulfan, in a ratio of 7:3 (Kataoka et al. 2010; Singh and Singh 2011). These stereo isomers are widely distributed in the environment and can be found in soil and sediments over long distances from the direct source of application.

The endosulfan half-life in soils is estimated to range from 60 to 800 days. However, another report has suggested half-lives for the combined toxic residues (endosulfan plus endosulfan sulfate) between 9 months and 6 years. Endosulfan can be released into the atmosphere through spray drift, post application volatilization, and wind erosion of soil particles. Both endosulfan and endosulfan sulfate were included in the Stockholm Convention list of persistent organic pollutants (POPs) in 2011. Because of its widespread use and distribution in the environment, endosulfan contaminates soils, groundwater, and sediments (Hussain et al. 2007a, b; Kumar and Philip 2007; Weber et al. 2009; Kalyani et al. 2010; Kataoka et al. 2010; Castillo et al. 2011; Singh and Singh 2011).

Endosulfan is especially toxic to insects (Wan et al. 2005) as well as fish and aquatic invertebrates (Dang et al. 2011) and causes endocrine disruption in mam-

R. Kondo (✉)
Kyushu University, 1-23-8 Kashii, Fukuoka, Japan
e-mail: ryukondo@agr.kyushu-u.ac.jp

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_7

mals (Singh et al. 2010). These health and environmental concerns have compelled us in developing ecofriendly, safe and economical alternatives for the detoxification of endosulfan in soil/water matrices.

2 Conversion of Endosulfan to Endosulfan Sulfate

A proportion of the residual endosulfan in soil is oxidized to endosulfan sulfate by indigenous microorganisms. Endosulfan sulfate is a toxic metabolite that is equally persistent, or more persistent than, the parent compound isomer (Abraham and Silambarasan 2014b). Endosulfan sulfate is the only breakdown product which considered as toxic intermediate (Berntssen et al. 2008). In the environment, endosulfan sulfate is the most frequently detected residue, particularly in soils and plant and animal tissues (Kumar and Philip 2007; Kataoka et al. 2010; Castillo et al. 2011). It is, therefore, very important that endosulfan sulfate is completely degraded to achieve the bioremediation of soils that have a history of endosulfan application.

3 Toxicity of Endosulfan

Several studies have demonstrated the toxicity of endosulfan. The toxicity of endosulfan is closely associated with the oxidative stress via generation of endosulfan-dependent reactive oxygen species (Sohn et al. 2004). This chemical has been found to be extremely toxic to fish (Ballesteros et al. 2007; Velasco-Santamaría et al. 2011) and is also recognized as an endocrine disruptor in amphibians (Hussain et al. 2007a; Kataoka et al. 2010). Besides, it is highly toxic to aquatic invertebrates (Chaudhuri et al. 1999). Endosulfan exposure used to affect both the reproductive and endocrine systems of experimental animals and humans (Dalsenter et al. 2003). Moreover, it has been reported that endosulfan causes neurotoxicity (Zhenquan and Hara 2007; Silva de Assis et al. 2011). In general, one of the main mechanisms underlying endosulfan toxicity is the induction of several genotoxic insults, including chromosomal aberrations in peripheral lymphocytes in field workers (Rupa et al. 1989, 1991; Carbonell et al. 1995), single strand breaks in freshwater fishes (Pandey et al. 2006; Sharma et al. 2007) and micronucleus induction in tadpole erythrocytes (Lajmanovicha et al. 2005). Besides, exposure of the earthworm *Eisenia fetida* to different doses of endosulfan produced DNA strand breaks (Liu et al. 2009). Wessel et al. (2007) found genotoxic damage in embryos of *Crassostrea gigas* exposed to increasing concentrations of endosulfan which resulted in DNA chain breakage. Neuparth et al. (2006) reported that endosulfan caused chromosomal damage in goldfish (*Sparus aurata*) found in water contaminated from agricultural soil runoff. Sharma et al. (2007) determined that endosulfan caused double and single DNA breaks, adduct formation, and DNA-DNA and DNA-protein cross-linking without leading to death in *Mystus vittatus*.

The genotoxic effects of endosulfan in humans have not been widely studied. However, acute and chronic toxicity of endosulfan is well known in a variety of mammals including human beings (Lu et al. 2000). Using comet assays, Bajpayee et al. (2006) found an increase in genetic damage in human lymphocytes exposed to endosulfan, endosulfan lactone, and endosulfan sulfate. Occupational exposure was also studied by Topé and Rogers (2009), who described the effects in humans occupationally exposed to a mixture of pesticides, including endosulfan. Endosulfan genotoxicity was also analyzed in HepG2 cells, and breaks of the DNA chain were clearly observed (Yuquan et al. 2000).

4 Bioremediation of Endosulfan

Biodegradation and bioremediation are interlinked processes to an extent that both of these are based on the conversion or metabolism of pesticides by microorganisms. The difference between these two is that, the biodegradation is a natural process, whereas bioremediation is an ecofriendly technology driven by microbes. In bioremediation, we use microbes to degrade the pesticides in situ, conditions while bioremediation technique requires an efficient bacterial strain that can degrade pollutant to a minimum level. Adequate rate of biodegradation is required to attain the acceptable level of pesticide residues or its metabolites at contaminated site in a limited time frame.

The rate of biodegradation in soil depends on four variables i.e. (i) availability of pesticide or metabolite to the microorganisms, (ii) physiological status of the microorganisms, (iii) survival and/or proliferation of pesticide degrading microorganisms at contaminated site and (iv) sustainable population of these microorganisms. Therefore, for successful bioremediation, it requires the creation of unique niche/or microhabitats for desired microbes, so that they can be successfully exploited. However, the difficulty is the limited knowledge about the population dynamics of pesticide degrading microorganisms in relation to other microbes present in the same habitat. Temperature, pH, water potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factors for pesticide degrading microorganisms, which require further exploration in relation to total microbial population and their biochemical activities.

For consideration of suitable bioremediation technique, the understanding of pesticide dynamics in soil environment is also required, as soil has unique binding potential for variety of pesticides or metabolites. It is also necessary to understand the physiology and genetics of bacterium for degradation of a particular pollutant (Singh 2008). Taking into account the extent of use and the high occurrence of endosulfan in the environment, as well as the hazardous risk of endosulfan exposure to humans and wildlife, it is necessary to develop both in situ and ex situ treatment techniques that enhance endosulfan degradation in the environment.

Endosulfan contamination has been detected in soil, water, air and food products because of its abundant usage and potential for environmental transport (Baig et al.

2009). Therefore, several strategies including bioremediation are being adopted to decontaminate the soil from such residual pollutants (Sutherland et al. 2000; Awasthi et al. 2003).

The use of organisms, isolated from contaminated sites, allows the development of biologically efficient and low-cost methods for the treatment of xenobiotic compounds. Therefore, the detoxification of endosulfan through biological means is receiving serious attention as an alternative to the existing methods, such as incineration and landfill (Siddique et al. 2003).

Endosulfan can be utilized as a sole source of carbon and/or sulfur during biodegradation to obtain non-toxic or less toxic endosulfandiol or oxidation to equally or more toxic endosulfan sulfate (Guerin 1999; Sutherland et al. 2000; Kumar and Philip 2006). Therefore, among the various cleanup technologies available, the biodegradation process, as opposed to other physic-chemical methods, is widely recognized as an effective bioremediation technique, as microorganisms grow in different ecosystems and are capable of surviving under adverse conditions due to co-existence with xenobiotic (Osman et al. 2008; Hussain et al. 2009; Krishna and Philip 2011).

After applying a pesticide degradation process using microbes, it is necessary to analyze a decrease in the pesticide concentration in the culture medium and to assess the decrease in toxicity. This assessment can be accomplished through the use of short-term tests, which provide information on the level of DNA damage caused by a genotoxin. In this context, the alkaline single-cell gel electrophoresis assay, which is also known as the comet assay, is a sensitive and reliable method for detecting alkali-labile and delayed repair sites, which are measured as DNA single-strand breaks, in eukaryotic individual cells. The comet assay is considered as an early biomarker of a biological effect and is widely used to assess DNA damage, both in vivo and in vitro conditions (Rojas et al. 1996; Valverde et al. 1997; Mussali-Galante et al. 2013).

5 Biodegradation of Endosulfan and Endosulfan Sulfate

Several intensive studies on the biodegradation of endosulfan in soils or in water environments, have been conducted using pure- or mixed-cultures of microorganisms (Kullman and Matsumura 1996; Sutherland et al. 2002b, c; Awasthi et al. 2003, 1997; Lee et al. 2003; Siddique et al. 2003; Sethunathan et al. 2004). Major biotransformation products of endosulfan include endosulfan sulfate and endosulfandiol, and between two products, endosulfan sulfate is more toxic and more persistent than the parent endosulfan (Kaur et al. 1998). Therefore, the research has focused on degradation of endosulfan sulfate to reduce the toxicity of soil (Sutherland et al. 2002c; Sethunathan et al. 2004).

Several microorganisms, capable of degrading endosulfan and some of its isomers, have been reported. Most of the biodegradation occurs at the sulfur moiety with no reports of dechlorination at the hexachloro portion of the structure

(Sutherland et al. 2002b). The pathway for endosulfan degradation via hydrolysis and formation of abiotic metabolites has been described (Sutherland et al. 2002b).

The biodegradation of endosulfan in soil and water environments by indigenous microbes has been reported by several workers such as *Achromobacter xylosoxidans* strain C8B (Singh and Singh 2011), *Bordetella* sp. B9 (Goswami and Singh 2009), *Klebsiella oxytoca* (Kwon et al. 2005), *Bacillus* sp. (Awasthi et al. 2003), *Pandoraea* sp. (Siddique et al. 2003), *Micrococcus* sp. (Guha et al. 2000), *Aspergillus niger* (Bhalerao and Puranik 2007), *Aspergillus terreus* and *Cladosporium oxysporum* (Mukherjee and Mittal 2005), *Fusarium ventricosum* (Siddique et al. 2003), *Mucor thermohyalospora* (Shetty et al. 2000), *Phanerochaete chrysosporium* (Kullman and Matsumura 1996), *Trichoderma harzianum* (Katayama and Matsumura 1993).

However, production of endosulfan sulfate is the major concern as this metabolite is more toxic, persists longer in soils and has bioaccumulation potential (Sutherland et al. 2002b). *Klebsiella oxytoca* (Kwon et al. 2005), *Bacillus* spp. (Awasthi et al. 2003), *Pandoraea* sp. (Siddique et al. 2003), and *Micrococcus* sp. (Guha et al. 2000) are the bacteria reported to degrade endosulfan in both solutions and soils. Many fungi have been also studied for their ability to degrade endosulfan, such as *Aspergillus niger* (Mukherjee and Gopal 1994), *Aspergillus terreus*, *Cladosporium oxysporum* (Mukherjee and Mittal 2005), *Mucor thermohyalospora* (Shetty et al. 2000), *Fusarium ventricosum* (Siddique et al. 2003), *Phanerochaete chrysosporium* (Kullman and Matsumura 1996), *Trichoderma harzianum* (Katayama and Matsumura 1993). Besides, algae, such as *Anabaena* sp. (Lee et al. 2003), *Chlorococcum* sp., and *Scenedesmus* sp. (Sethunathan et al. 2004), are the photosynthetic microorganisms which were used in endosulfan degradation studies.

5.1 Degradation by Bacteria

Endosulfan degradation by microorganisms has been studied mainly with bacteria isolated from soils contaminated with pesticides over long periods of time. Some bacterial species which were found capable of degrading endosulfan are *Klebsiella pneumonia, Pseudomonas spinosa, Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia, Rhodococcus erythropolis, Achromobacter xylosoxidans*, *Mycobacterium* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Bordetella* sp. and *Pseudomonas* sp. (Kwon et al. 2002; Sutherland et al. 2002a; Lee et al. 2006; Weir et al. 2006; Hussain et al. 2007a; Kumar and Philip 2007; Goswami et al. 2009; Bajaj et al. 2010; Singh and Singh 2011).

Kumar et al. (2008) showed that *Ochrobacterum* sp., *Arthrobacter* sp. and *Burkholderia* sp. degraded α- and β-endosulfan by over 60 % in soil. Arshad et al. (2008) also reported that *Pseudomonas aeruginosa* was removable to more than 85 % of α- and β-endosulfan under soil slurry conditions. Endosulfan can be used by the bacteria as a sole carbon, sulfur and energy source in both water or soil matrices (Sutherland et al. 2000; Lee et al. 2006; Li et al. 2009; Verma et al. 2011).

Bacteria, such as *Arthrobacter* sp. (Kumar et al. 2008), *Burkholderia cepacia* (Hussain et al. 2007a), *Pseudomonas* sp. (Arshad et al. 2008) and *Rhodococcus* sp. (Verma et al. 2011) were widely used in the majority of biodegradation and bioremediation endosulfan studies, and their biodegradation potential is unmatched.

Many pure bacterial species have been identified for the formation of one or more metabolites using endosulfan isomers as sole source of sulfur (Katayama and Matsumura 1993; Mukherjee and Gopal 1994; Kullman and Matsumura 1996), with an exception of a bacterial co-culture utilizing endosulfan as a source of carbon (Awasthi et al. 1997). A few gram-positive bacteria, such as *Bacillus* species from endosulfan-contaminated industrial soil, were characterized for the conversion of endosulfan into endosulfan sulfate (Shivaramaiah and Kennedy 2006). Kwon et al. (2002) reported *Klebsiella pneumoniae* KE-1, a potential endosulfan degrader, while, *Klebsiella oxytoca* KE-8 had high potential as a biocatalyst for the bioremediation of endosulfan and/or endosulfan sulfate (Kwon et al. 2005).

Endosulfan and their metabolites were detected in soils with a history of endosulfan application. *Enterobacter asburiae* JAS5 and *Enterobacter cloacae* JAS7 were isolated, and these bacterial strains were found as potential candidates for the bioremediation of pesticide-contaminated agricultural fields (Abraham and Silambarasan 2015). Kumar and Philip (2007) studied the degradation of endosulfan by three bacterial species, namely *Staphylococcus* sp., *Bacillus circulans*-I, and *Bacillus circulans*-II, both in mixed culture and pure culture. In mixed culture, after four weeks of incubation, degradation of 71.8 ± 0.2 % and 76.0 ± 0.2 % of endosulfan was observed in aerobic and facultative anaerobic conditions, respectively.

5.2 *Metabolism of Degradation of Endsulfan and Endsulfan Sulfate*

In general, two major degradation routes (oxidative and hydrolytic) for endosulfan have been elucidated in different organisms. The degradation is specific to α- and β-isomer, and their catalytic rates also vary significantly (Kwon et al. 2005).

A few bacterial and fungal strains, that degrade endosulfan isomers, have been isolated and characterized in many laboratories (Siddique et al. 2003; Hussain et al. 2007a). Microbial metabolism of endosulfan often results in the formation of a toxic endosulfan sulfate via oxidation and a less toxic endosulfandiol by hydrolysis (Kullman and Matsumura 1996; Sutherland et al. 2000). Endosulfandiol can be further transformed into endosulfan ether, endosulfan hydroxyl ether, endosulfan-dialdehyde and endosulfan lactone, which are less toxic metabolites (Sutherland et al. 2002b, c). However, degradation product, endosulfan sulfate, has been found toxic to biomolecules (Siddique et al. 2003). Kwon et al. (2002)isolated a soil bacterium *Klebsiella pneumoniae* KE-1 that degrades endosulfan without formation of the toxic metabolite endosulfan sulfate.

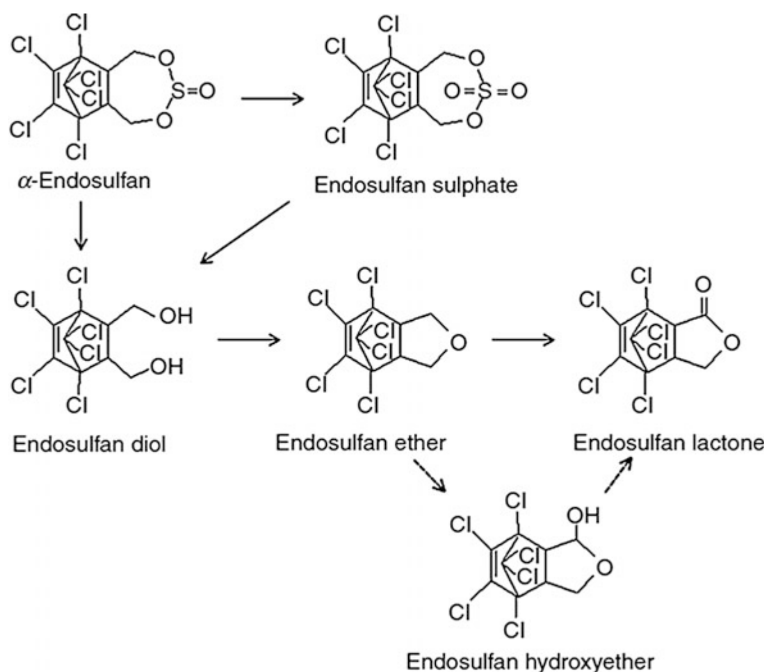


Fig. 1 Proposed pathway for metabolism of α-endosulfan (ES) and ES sulfate by the *Pseudomonas* sp. strain IITR01. *Dotted lines* indicate the possibility of ES lactone formation via hydroxyether (not detected in analysis) (Bajaj et al. 2010)

Other studies on the microbial degradation of endosulfan have also revealed formation of various intermediates during metabolism, such as endosulfan sulfate, endosulfandiol, endosulfan ether, endosulfan lactone, endosulfanhydroxyether and endosulfandialdehyde (Martens 1976; Kwon et al. 2005). *Achromobacter xylosoxidans* strain CS5 was able to utilize both endosulfan and endosulfan sulfate as sulfur as well as carbon and energy sources, leading to complete mineralization of endosulfan via hydrolytic pathway (Wen et al. 2009). Similarly, a *Pseudomonas* sp. strain IITR01 was found capable of degrading α-endosulfan and toxic endosulfan sulfate. The analytical data showed the disappearance of both α-endosulfan and endosulfan sulfate and the formation of hydroxylated products endosulfandiol, ether and lactone (Bajaj et al. 2010) (Fig. 1).

The degradation of endosulfan metabolites i.e., endosulfan sulfate, endosulfan ether and endosulfan lactone was reported in the aerobic conditions. The degradation of enodulfan ether and endosulfan lactone was carried out by *Bacillus circulans*I and II whereas no endosulfan sulfate was degraded by any of these strains. Endosulfan ether was further converted to endosulfan lactone and some other intermediate compounds (Kumar and Philip 2007).

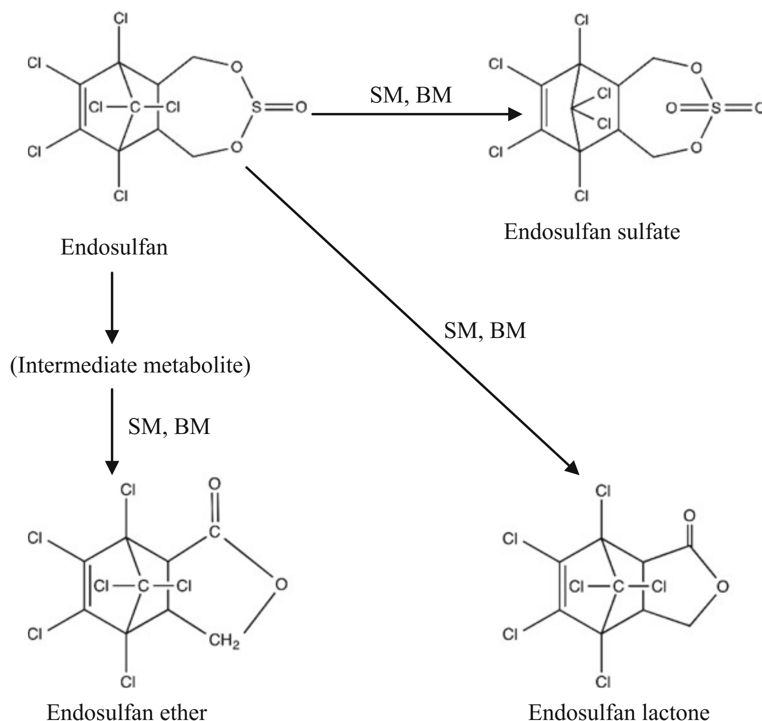


Fig. 2 Endosulfan degradation by *Aspergillus sydoni* in broth medium and soil microcosm. *SM* soil microcosm, *BM* broth medium (Goswami et al. 2009)

Several pathways for fungal metabolism of endosulfan were proposed (Kullman and Matsumura 1996; Siddique et al. 2003; Bhalerao and Puranik 2007; Goswami et al. 2009). Fungal oxidative metabolism of endosulfan most often results in the formation of endosulfansulphate, which is as toxic and persistent as the parent compound. Endosulfandiol is a major product of endosulfan hydrolysis and can further be slowly degraded to less toxic endosulfan ether, endosulfanhydroxyether and endosulfan lactone (Kullman and Matsumura 1996; Goswami et al. 2009) (Fig. 2).

5.3 *Microbial Enzymes and Gene*

It is important to note that some microbial enzymes are specific to one isomer, or catalyse each isomer at different rates. For example, a *Mycobacterium tuberculosis* endosulfan degradation enzyme degrades β-endosulfan to the mono-aldehyde and hydroxyether, but transforms α-endosulfan to the more toxic endosulfan sulfate (Sutherland et al. 2002b, c). On the contrary, oxidation of endosulfan or endosulfan

sulfate by the mono-oxygenase encoded by *ese* in *Arthrobacter* sp. KW yields endosulfan mono-alcohol (Weir et al. 2006). Both proteins encoded by *ese* and *esd* are part of the unique two component flavin-dependent monooxygenase family, which require reduced flavin (Sutherland et al. 2002a).

The metabolizing potential of a bacterial strain *Rhodococcus* MTCC 6716, isolated from the gut of an Indian earthworm (*Metaphire posthuma*), was studied for endosulfan bioremediation. Endosulfan-induced alteration in the expression of mRNA and protein of specific endosulfan metabolizing marker gene (Esd) was studied. Endosulfan degradation was mediated through gene(s) present in genomic DNA. Expression of marker gene was found to be endosulfan concentration dependent. The results suggest that this novel strain (*Rhodococcus* MTCC 6716) may be utilized for bioremediation of endosulfan (Verma et al. 2011).

Metabolism of α-endosulfan and endosulfate was also observed using the crude cell extract of *Pseudomonas* sp. strain IITR01. The molecular mass of protein, induced during the degradation of α-endosulfan and sulfate as substrate, was found to be approximately 150 kDa (Bajaj et al. 2010).

5.4 Degradation by Filamentous Fungi

Studies of endosulfan degradation by filamentous fungal organisms are very scarce. Fungal organisms have advantages over the bacterial strains, e.g., the fungi enzymes of the lignocellulolytic complex have been related to the degradation of various xenobiotic pollutants, including pesticides. The disadvantages of some fungal strains include the growth and degradation times. For example, Bhalerao and Puranik (2007) achieved endosulfan degradation using *Aspergillus niger*, but the complete mineralization process required a period of 12 days. *Phanerochaete chrysosporium, Aspergillus terreus*, *Aspergillus terricola, Chaetosartoryas tromatoides, Mortierella* sp., *Trametes hirsute*, and *Mucor thermohyalospora* were also reported to remove 50–90 % of endosulfan over a period of 12–28 days (Kullman and Matsumura 1996; Shetty et al. 2000; Siddique et al. 2003; Hussain et al. 2007b; Elsaid et al. 2010; Kataoka et al. 2010; Kamei et al. 2011).

A screening of fungi capable of metabolizing endosulfan was carried out. On the basis of tolerance to endosulfan, an isolate, identified as *Aspergillus niger* was selected. Complete disappearance of endosulfan was observed with the formation of various intermediates of endosulfan metabolism including endosulfandiol, endosulfan sulfate, and an unidentified metabolite. The toxic intermediate, endosulfan sulfate, was also further metabolized, resulting in complete mineralization of endosulfan (Bhalerao and Puranik 2007).

The biodegradation of endosulfan and the metabolites formed was also studied using a fungus *Aspergillus sydoni.* Endosulfan sulfate was the main metabolite formed along with small quantity of endosulfan ether and endosulfan lactone during endosulfan degradation (Goswami and Singh 2009). Two different reports indicated that *Phanerochaete chrysosporium* was able to degrade endosulfan into four

metabolites (Kullman and Matsumura 1996), while a *Fusarium* fungus metabolized both the α- and β-endosulfan have (Siddique et al. 2003). The ability of two white-rot fungi (*Trametes versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus (*Gloeophyllum trabeum*) was assessed to degrade two organochlorine insecticides, lindane and endosulfan. The amount of degraded lindane and endosulfan increased correspondingly with their exposure period in the liquid cultures of both examined white-rot fungi. Endosulfan was transformed to endosulfan sulphate by both *T. versicolor* and *P. ostreatus*. Besides, a small amount of endosulfan ether was also detected (Ulčniket al. 2013).

There are a few reports available on the degradation of endosulfan in soil by fungi, Goswami et al. (2009) reported that *Aspergillus sydoni* had the ability to degrade endosulfan in unsterilized soil. Therefore, endosulfan degradation in soil is still inadequately explored.

The *Penicillium* sp. CHE 23 strain can be used to degrade endosulfan residues and/or in water and soil bioremediation processes without causing any toxicity problems, which are probably due to the formation of non-toxic metabolites during biodegradation (Romero-Aguilar et al. 2014).

The bioremediation potential of *Mortierella* sp. strain W8 in endosulfan-contaminated soil was examined. Endosulfandiol metabolite was detected. Production of toxic endosulfan sulfate, the main metabolite of endosulfan, was suppressed by this fungal treatment (Kataoka et al. 2011).

5.5 Degradation by Bacterial and Fungal Consortiums

Though there are many reports pertaining to degradation of endosulfan with single isolate, not much work has been done with bacterial or fungal consortium. A microbial consortium consisting of bacterial strains and fungal strains were originally obtained from endosulfan contaminated agricultural soils. Identification of the bacterial isolates by 16S rRNA sequences revealed the isolates to be *Halophilic bacterium* JAS4, *Klebsiella pneumonia* JAS8, *Enterobacter asburiae* JAS5, and *Enterobacter cloacae* JAS7, whereas the fungal isolates were identified by 18S rRNA sequences and the isolates were *Botryosphaeria laricina* JAS6, *Aspergillus tamari* JAS9 and *Lasiodiplodia* sp. JAS12. The bacterial and fungal consortium could degrade 1000 mg $l^{-1}$of endosulfan efficiently in aqueous medium and in soil (Abraham and Silambarasan 2014a).

6 Future Prospectives

Pesticide degradation has attracted the attention of several scientists in recent years. A major concern associated with use of bioremediation in the field is the applicability of laboratory research to actual large-scale contaminated field sites. The true

test of any bioremediation technology is in the field application. Because of high costs involved in field application, only a few pilot and full-scale studies using microorganisms for biodegradation are only available in the literature. The majority of the research on microbial performance has been conducted in vitro conditions either in sterile soil or synthetic media. While the results of several works revealed that microorganisms were highly capable to degrade toxicorgano-pollutants in these conditions, but its field application did not show promising results due to variable environmental conditions.

7 Conclusions

The inherent capability of many soil microbes to degrade pesticides have been explored, but still use of these organisms in the field remains a big issue for the bioremediation process. Bioavailability is one of the important limitations which affect the in situ bioremediation process. Introducing the soil enriched with capable microbes back to the contaminated environment still remains a problem because of the variation in the environmental factors. In most cases, microbes are easily susceptible to the variation in temperature, pH, moisture content, nutrients and also by competitive inhibition caused by other microbes in the environment. These factors along with non-bioavailable fraction of the soil-bound pesticides adversely affect the bioremediation process. Even though many ex situ treatment technologies have been developed, the process requires a large scale excavation of soil which increases the cost. Moreover, most of these organisms are not able to degrade highly toxic metabolites formed during parent compound degradation and also due to other toxicants present in the field. Hence, an integrated study on bioremediation of the pesticides is needed for developing an efficient treatment technology based on the field and laboratory studies. In the field, ageing of the pesticides makes it to sequester into soil Nano pores where they become unavailable for the microbes to degrade. Hence, research should not only focus on integrated approach, but also on enhancing the bioavailability of matrix-bound pesticide residues and its metabolites.

References

Abraham, J., & Silambarasan, S. (2014a). Biomineralization and formulation of endosulfan degrading bacterial and fungal consortiums. *Pesticide Biochemistry and Physiology, 116*, 24–31.

Abraham, J., & Silambarasan, S. (2014b). Role of novel fungus *Lasiodiplodia* sp. JAS12 and plant growth promoting bacteria *Klebsiella pneumoniae* JAS8 in mineralization of endosulfan and its metabolites. *Ecological Engineering, 70*, 235–240.

Abraham, J., & Silambarasan, S. (2015). Plant growth promoting bacteria *Enterobacter asburiae* JAS5 and *Enterobacter cloacae* JAS7 in mineralization of endosulfan. *Applied Biochemistry and Biotechnology, 175*, 3336–3348.

Arshad, M., Hussain, S., & Saleem, M. (2008). Optimization of environmental parameters for biodegradation of alpha and beta endosulfan in soil slurry by *Pseudomonas aeruginosa*. *Journal of Applied Microbiology, 104*, 364–370.

Awasthi, N., Manickam, N., & Kumar, A. (1997). Biodegradation of endosulfan by a bacterial coculture. *Bulletin of Environmental Contamination and Toxicology, 59*, 928–934.

Awasthi, N., Singh, A. K., Jain, R. K., Khangrot, B. S., & Kumar, A. (2003). Degradation and detoxification of endosulfan isomers by a defined co-culture of two *Bacillus* strains. *Applied Microbiology and Biotechnology, 62*, 279–283.

Baig, S. A., Akhtera, A. N., Ashfaq, M., & Asi, M. R. (2009). Determination of the organophosphorus pesticide in vegetables by high-performance liquid chromatography. *American-Eurasian Journal of Agriculture and Environmental Science, 6*(5), 513–519.

Bajaj, A., Pathak, A., Mudiam, M. R., Mayilraj, S., & Manickam, N. (2010). Isolation and characterization of a *Pseudomonas* sp. strain IITR01 capable of degrading α-endosulfan and endosulfan sulfate. *Journal of Applied Microbiology, 109*, 2135–2143.

Bajpayee, M., Kumar, A. P., Zaidi, S., Musarrat, J., Parmar, D., Mathur, N., et al. (2006). DNA damage and mutagenicity induced by endosulfan and its metabolites. *Environmental and Molecular Mutagenesis, 47*, 682–692.

Ballesteros, M. L., Bianchi, G. E., Carranza, M., & Bistoni, M. A. (2007). Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). *Journal of Environmental Science and Health Part B, 42*, 351–357.

Berntssen, M. H., Glover, C. N., Robb, D. H., Jakobsen, J. V., & Petri, D. (2008). Accumulation and elimination kinetics of dietary endosulfan in Atlantic salmon (*Salmos alar*). *Aquatic Toxicology, 86*, 104–111.

Bhalerao, T. S., & Puranik, P. R. (2007). Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. *International Biodeterioration and Biodegradation, 59*, 315–321.

Carbonell, E., Valbuena, A., Xamena, N., Creus, A., & Marcos, R. (1995). Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides. *Mutation Research/Genetic Toxicology, 344*(3), 127–134.

Castillo, J. M., Casas, J., & Romero, E. (2011). Isolation of an endosulfan-degrading bacterium from a coffee farm soil: Persistence and inhibitory effect on its biological functions. *Science of the Total Environment, 412–413*, 20–27.

Chaudhuri, K., Selvaraj, S., & Pal, A. K. (1999). Studies on the genotoxicity of endosulfan in bacterial systems. *Mutation Research, 439*, 63–67.

Dalsenter, P. R., Silva de Assis, H. C., Araújo, S. L., Andrade, A. J. M., & Dallegrave, E. (2003). Pre and postnatal exposure to endosulfan in Wistar rats. *Human and Experimental Toxicology, 22*, 171–175.

Dang, Z., Ru, S., Wang, W., Rorije, E., Hakkert, B., & Vermeire, T. (2011). Comparison of chemical-induced transcriptional activation of fish and human estrogen receptors: regulatory implications. *Toxicology Letters, 201*, 152–175.

Elsaid, O. E. G., Abdelbagi, A. O., & Elsheikhc, E. A. E. (2010). Microbial degradation of endosulfan in carbon free media and selective media. *Research Journal of Agriculture Biological Sciences, 6*(3), 257–262.

Goswami, S., & Singh, D. K. (2009). Biodegradation of α and β endosulfan in broth medium and soil microcosm by bacterial strain *Bordetella* sp. B9. *Biodegradation, 20*, 199–207.

Goswami, S., Vig, K., & Singh, D. K. (2009). Biodegradation of α and β endosulfan by *Aspergillus sydoni*. *Chemosphere, 75*, 883–888.

Guerin, T. F. (1999). The anaerobic degradation of endosulfan by indigenous microorganisms from low-oxygen soils and sediments. *Environmental Pollution, 106*(1), 13–21.

Guha, A., Kumari, B., Bora, T. C., Deka, P. C., & Roy, M. K. (2000). Bioremediation of endosulfan by *Micrococcus* sp. *Indian Journal of Environmental Health, 42*, 9–12.

Hatipoglu, F. S., Gulay, M. S., Balic, A., Yildiz-Gulay, O., & Volkan, S. (2008). Subacute oral toxicity of endosulfan in male New Zealand white rabbits. *Toxicology Mechanisms and Methods, 18*, 705–710.

Hussain, S., Arshad, M., Saleem, M., & Khalid, A. (2007a). Biodegradation of alpha and beta-endosulfan by soil bacteria. *Biodegradation, 18*, 731–740.

Hussain, S., Arshad, M., Saleem, M., & Zahir, Z. A. (2007b). Screening of soil fungi for *in vitro* degradation of endosulfan. *World Journal of Microbiology & Biotechnology, 23*, 939–945.

Hussain, S., Siddique, T., Arshad, M., & Saleem, M. (2009). Bioremediation and phytoremediation of pesticides: recent advances. *Critical Reviews in Environmental Science and Technology, 39*, 843–907.

Kalyani, S. S., Sharma, J., Dureja, P., & Singh, S. L. (2010). Influence of endosulfan on microbial biomass and soil enzymatic activities of a tropical alfisol. *Bulletin of Environmental Contamination and Toxicology, 84*, 351–356.

Kamei, I., Takagi, K., & Kondo, R. (2011). Degradation of endosulfan and endosulfan sulfate by white-rot fungus *Trametes hirsuta*. *Journal of Wood Science, 57*, 317–322.

Kataoka, R., Takagi, K., & Sakakibara, F. (2010). A new endosulfan degrading fungus, *Mortierella* species, isolated from a soil contaminated with organochlorine pesticides. *Journal of Pesticide Science, 35*(3), 326–332.

Kataoka, R., Takagi, K., & Sakakibara, F. (2011). Biodegradation of endosulfan by *Mortieralla* sp. strain W8 in soil: Influence of different substrates on biodegradation. *Chemosphere, 85*, 548–552.

Katayama, A., & Matsumura, F. (1993). Degradation of organochlorine pesticides, particularly endosulfan by *Trichoderma harzianum*. *Environmental Toxicology and Chemistry, 12*, 1059–1065.

Kaur, I., Mathur, R. P., Tandon, S. N., & Dureja, P. (1998). Persistence of endosulfan (technical) in water and soil. *Environmental Technology, 19*, 115–119.

Krishna, R. K., & Philip, L. (2011). Bioremediation of single and mixture of pesticide-contaminated soils by mixed pesticide-enriched cultures. *Applied Biochemistry and Biotechnology, 2011*, 1–21.

Kullman, S. W., & Matsumura, F. (1996). Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan. *Applied and Environmental Microbiology, 62*, 593–600.

Kumar, M., Lakshmi, C. V., & Khanna, S. (2008). Biodegradation and bioremediation of endosulfan contaminated soil. *Bioresource technology, 99*, 3116–3122.

Kumar, M., & Philip, L. (2006). Endosulfan mineralization by bacterial isolates and possible degradation pathway identification. *Bioremediation Journal, 10*(4), 179–190.

Kumar, M., & Philip, L. (2007). Biodegradation of endosulfan contaminated soil in a pilot-scale reactor bioaugmented with mixed bacterial culture. *Journal of Environmental Science and Health Part B, 42*, 707–715.

Kwon, G. S., Kim, J. E., Kim, T. K., Sohn, H. Y., Koh, S. C., Shin, K. S., et al. (2002). *Klebsiella pneumoniae* KE-1 degrades endosulfan without formation of the toxic metabolite, endosulfan sulfate. *FEMS Microbiology Letters, 215*, 255–259.

Kwon, G. S., Sohn, H. Y., Shin, K. S., Kim, E., & Seo, B. (2005). Biodegradation of the organochlorine insecticide, endosulfan, and the toxic metabolite, endosulfan sulfate, by *Klebsiella oxytoca* KE-8. *Applied Microbiology and Biotechnology, 67*, 845–850.

Lajmanovicha, R. C., Cabagnab, M., Peltzera, P. M., Stringhinic, G. A., & Attademoc, A. M. (2005). Micronucleus induction in erythrocytes of the *Hylapulchella tadpoles* (Amphibia: Hylidae) exposed to insecticide endosulfan. *Mutation Research, 587*, 67–72.

Lee, S.-E., Kim, J.-S., Kennedy, I. R., Park, J.-W., Kwon, G.-S., Koh, S.-C., et al. (2003). Biotransformation of an organochlorine insecticide, endosulfan, by *Anabaena* species. *Journal of Agricultural and Food Chemistry, 51*, 1336–1340.

Lee, J. B., Sohn, H. Y., Shin, K. S., Jo, M. S., Kim, J. E., Lee, S. W., et al. (2006). Isolation of a soil bacterium capable of biodegradation and detoxification of endosulfan and endosulfan sulfate. *Journal of Agricultural and Food Chemistry, 54*, 8824–8828.

Li, W., Dai, Y., Xue, B., Li, Y., Peng, X., & Zhang, J. (2009). Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5. *Journal of Hazardous Materials, 167*, 209–216.

Liu, W., Zhu, L. S., Wang, J., Wang, J. H., Xie, H., & Song, Y. (2009). Assessment of the genotoxicity of endosulfan in earthworm and white clover plants using the comet assay. *Archives of Environmental Contamination and Toxicology, 56*, 742–746.

Lu, Y., Morimoto, K., Takeshita, T., Takeuchi, T., & Saito, T. (2000). Genotoxic effects of α-endosulfan and β-endosulfan on human HepG2 cells. *Environmental Health Perspectives, 108*, 559–561.

Martens, R. (1976). Degradation of [8,9-^{14}C] endosulfan by soil microorganisms. *Applied and Environmental Microbiology, 31*, 853–858.

Mukherjee, I., & Gopal, M. (1994). Degradation of beta-endosulfan by *Aspergillus niger*. *Toxicological and Environmental Chemistry, 46*, 217–221.

Mukherjee, I., & Mittal, A. (2005). Bioremediation of endosulfan using *Aspergillus terreus* and *Cladosporium oxysporum*. *Bulletin of Environmental Contamination and Toxicology, 75*, 1034–1040.

Mussali-Galante, P., Tovar-Sánchez, E., Valverde, M., & Rojas, C. E. (2013). Biomarkers of exposure for assessing environmental metal pollution: from molecules to ecosystems. *Revista Internacional de Contaminación Ambiental, 29*(1), 117–140.

Neuparth, T., Bickham, J. W., Theodorakis, C. W., Costa, F. O., & Costa, M. H. (2006). Endosulfan induced genotoxicity detected in the gilthead sea bream, *Sparus aurata* L., by means of flow cytometry and micronuclei assays. *Bulletin of Environmental Contamination & Toxicology, 76*, 242–248.

Osman, K. A., Ibrahim, G. H., Askar, A. I., & Aba Alkhail, A. R. A. (2008). Biodegradation kinetics of dicofol by selected microorganisms. *Pesticide Biochemistry and Physiology, 91*, 180–185.

Pandey, S., Nagpure, N. S., Kumar, R., Sharma, S., Srivastava, S. K., & Verma, M. S. (2006). Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single-cell gel electrophoresis. *Ecotoxicology and Environmental Safety, 65*, 56–61.

Rojas, E., Valverde, M., Sordo, M., & Ostrosky-Wegman, P. (1996). DNA damage in exfoliated buccal cells of smokers assessed by the single cell electrophoresis assay. *Mutation Research, 370*, 115–120.

Romero-Aguilar, M., Tovar-Sánchez, E., Sánchez-Salinas, E., Mussali-Galante, P., Sánchez-Meza, J. C., Castrejón-Godínez, M. L., et al. (2014). *Penicillium* sp. as an organism that degrades endosulfan and reduces its genotoxic effects. *SpringerPlus, 3*, 536–546.

Rupa, D. S., Reddy, P. P., & Reddi, O. S. (1989). Chromosomal aberrations in peripheral lymphocytes of cotton field workers exposed to pesticides. *Environmental Research, 49*, 1–6.

Rupa, D. S., Reddy, P. P., & Reddi, O. S. (1991). Clastogenic effect of pesticides in peripheral lymphocytes of cotton-field workers. *Mutation Research, 261*, 177–180.

Sethunathan, N., Megharaj, M., Chen, Z. L., Williams, B. D., Lewis, G., & Naidu, R. (2004). Algal degradation of a known endocrine disrupting insecticide, α-endosulfan, and its metabolite, endosulfan sulfate, in liquid medium and soil. *Journal of Agricultural and Food Chemistry, 52*, 3030–3035.

Sharma, S., Nagpure, N. S., Kumar, R., Pandey, S., Srivastava, S. K., Singh, P. J., Mathur, P. K. (2007). Studies on the genotoxicity of endosulfan in different tissues of fresh water fish *Mystus vittatus* using the comet assay. *Archives of Environmental Contamination and Toxicology*, l53, 617–623.

Shetty, P. K., Mitra, J., Murthy, N. B. K., Namitha, K. K., Savitha, K. N., & Raghu, K. (2000). Biodegradation of cyclodiene insecticide endosulfan by *Mucor thermohyalospora* MTCC 1384. *Current Science, 79*, 1381–1383.

Shivaramaiah, H. M., & Kennedy, I. R. (2006). Biodegradation of endosulfan by a soil bacterium. *Journal of Environmental Science and Health Part B, 41*, 895–905.

Siddique, T., Okeke, B. C., Arshad, M., & Frankenberger, W. T. (2003). Enrichment and isolation of endosulfan-degrading microorganisms. *Journal of Environmental Quality, 32*, 47–54.

Silva de Assis, H. S., Nicaretta, L., Andrade, M. C., Crestani, S., Soares, K., Olmedo, D., et al. (2011). Anticholinesterasic activity of Endosulfan in wistar Rats. *Bulletin of Environmental Contamination and Toxicology, 86*, 368–372.

Singh, D. K. (2008). Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. *Indian Journal of Microbiology, 48*, 35–40.

Singh, J. K., Ali, M., Kumar, R., Nath, A., & Kumar, A. (2010). Possible occurrence of apoptosis in endosulfan induced testicular toxicity in mice. *Journal of Ecophysiology and Occupational Health, 10*, 43–47.

Singh, N. S., & Singh, D. K. (2011). Biodegradation of endosulfan and endosulfan sulfate by *Achromobacter xylosoxidans* strain C8B in broth medium. *Biodegradation, 22*(5), 845–857.

Sohn, H.-Y., Kwon, C.-S., Kwon, G.-S., Lee, J.-B., & Kim, E. (2004). Induction of oxidative stress by endosulfan and protective effect of lipid- soluble antioxidants against endosulfan-induced oxidative damage. *Toxicology Letters, 151*, 357–365.

Sutherland, T. D., Horne, I., Harcourt, R. L., Russell, R. J., & Oakeshott, J. G. (2002a). Gene cloning and molecular characterization of a two-enzyme system catalyzing the oxidative detoxification of β-endosulfan. *Applied and Environmental Microbiology, 68*, 6237–6245.

Sutherland, T. D., Horne, I., Harcourt, R. L., Russell, R. J., & Oakeshott, J. G. (2002b). Isolation and characterization of a *Mycobacterium* strain that metabolizes the insecticide endosulfan. *Journal of Applied Microbiology, 93*, 380–389.

Sutherland, T. D., Horne, I., Lacey, M. J., Harcourt, R. L., Russell, R. J., & Oakeshott, J. G. (2000). Enrichment of an endosulfan–degrading mixed bacterial culture. *Applied and Environmental Microbiology, 66*, 2822–2828.

Sutherland, T. D., Weir, K. M., Lacey, M. J., Horne, I., Russell, R. J., & Oakeshott, J. G. (2002c). Enrichment of a microbial culture capable of degrading endosulphate, the toxic metabolite of endosulfan. *Journal of Applied Microbiology, 92*, 541–548.

Topé, A., & Rogers, V. M. (2009). Evaluation of protective effects of sulforaphane on DNA damage caused by exposure to low levels of pesticide mixture using comet assay. *Journal of Environmental Science and Health Part B, 44*, 657–662.

Ulčnik, A., Cigić, I. K., & Pohleven, F. (2013). Degradation of lindane and endosulfan by fungi, fungal and bacterial laccases. *World Journal of Microbiology & Biotechnology, 29*, 2239–2247.

Valverde, M., Lopez, M. C., Lopez, I., Sanchez, I., Fortoul, T. I., Ostrosky-Wegman, P., et al. (1997). DNA damage in leukocytes and buccal and nasal epithelial cells of individuals exposed to air pollution in Mexico City. *Environmental and Molecular Mutagenesis, 130*, 147–152.

Velasco-Santamaría, Y. M., Handy, R. D., & Sloman, K. A. (2011). Endosulfan affects health variables in adult zebrafish (*Daniorerio*) and induces alterations in larvae development. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 153*(4), 372–380.

Verma, A., Ali, D., Farooq, M., Pant, A. B., Ray, R. S., & Hans, R. K. (2011). Expression and inducibility of endosulfan metabolizing gene in *Rhodococcus* strain isolated from earthworm gut microflora for its application in bioremediation. *Bioresource technology, 102*, 2979–2984.

Wan, M. T., Kuo, J.-N., Buday, C., Schroeder, G., Van Aggelen, G., & Pasternak, J. (2005). Toxicity of α-, β-, (α + β)-endosulfan and their formulated and degradation products to *Daphnia magna*, *Hyalella azteca*, *Oncophynchus mykiss*, *Oncophynchus kisutch*, and biological implications in streams. *Environmental Toxicology and Chemistry, 24*, 1146–1154.

Weber, J., Halsall, C., Muir, D., Teixeira, C., Small, J., Solomon, K., et al. (2009). Endosulfan, a global pesticide: a review of its fate in the environment and occurrence in the Arctic. *Science of the Total Environment, 408*(15), 2966–2984.

Weir, K. M., Sutherland, T. D., Horne, I., Russell, R. J., & Oakeshott, J. G. (2006). A single monooxygenase, *ese*, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an *Arthrobacter* sp. *Applied and Environmental Microbiology, 72*, 3524–3530.

Wen, L., Yun, D., Beibei, X., Yingying, L., Xiang, P., Zhangg, J., et al. (2009). Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5. *Journal of Hazardous Materials, 167*, 209–216.
Wessel, N., Rousseau, S., Caisey, X., Quiniou, F., & Akcha, F. (2007). Investigating the relationship between embryotoxic and genotoxic effects of benzo[a], 17α-ethinyl estradiol and endosulfan on *Crassostrea gigas* embryos. *Aquatic Toxicology, 85*, 133–142.
Yuquan, L., Morimoto, K., Takeshita, T., Takeuchi, T., & Saito, T. (2000). Genotoxic effects of alpha-endosulfan and beta-endosulfan on human HepG2 cells. *Environmental Health Perspectives, 108*(6), 559–561.
Zhenquan, J., & Hara, P. M. (2007). Developmental exposure to pesticides zineb and/or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals later in life. *Neurotoxicology, 28*, 727–735.

Microbe Induced Degradation of Pesticides in Agricultural Soils

Durgesh Kumar Jaiswal, Jay Prakash Verma and Janardan Yadav

1 Introduction

The Green revolution was initiated with the development of agricultural tools and techniques that began in Mexico in 1944. In the green revolution, the use of chemical fertilizers and pesticides was enhanced to boost up food grain production to meet the needs of the ever increasing human population (Verma et al. 2014). Notwithstanding, the green revolution also caused many environmental problems that included loss of soil fertility, acidification, nitrate leaching, weed invasion and loss of biodiversity (Tilman et al. 2002; Verma et al. 2013). With the growing human population industrialization and urbanization were also enhanced which caused the accumulation of various chemicals (Porto et al. 2011). These chemicals caused a lot of environmental concern, like bio-magnifications in each tropic level of soil and water ecosystem. Various treatment methods are now used like land filling of contaminated site, recycling, pyrolysis and incineration for removing and remediation of pollution from the contaminated site. However, these methods are too costly and also not very effective to remediate the toxic chemicals. As 30 % of agricultural produce is lost due to pests, the use of pesticides has become indispensable in agriculture (Porto et al. 2011). However, the pesticide remediation from agriculture field is very different and also very expensive (Jain et al. 2005). However, the use of effective microorganisms to remove chemical and pesticide pollutants from contaminated sites (soil and water) is very promising. This treatment method is effective, less hazardous, economically viable, versatile and also

D.K. Jaiswal · J.P. Verma (✉)
Institute of Environment and Sustainable Development, Banaras Hindu University,
Varanasi 221005, Uttar Pradesh, India
e-mail: verma_bhu@yahoo.co.in; jpv.iesd@bhu.ac.in

J. Yadav
Department of Soil Science and Agricultural Chemistry, Institute of Agriculture
Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_8

environment-friendly. This process is known as microbial bioremediation (Finley et al. 2010). The genetic modified microorganism was also used to enhance the efficacy of bioremediation (Schroll et al. 2004).

No doubt, application of chemical pesticides has contributed greatly to the increase of yields in agriculture by controlling pests and diseases and also checking the insect-borne diseases (malaria, dengue, encephalitis, filariasis, etc.) caused to human beings (Bhatnagar 2001; Rekha and Naik 2006). Although effective pest management enhanced the agricultural production significantly, but 45 % of annual food production is lost by pest infestation. In tropical countries, crop loss is even more severe, because of high temperature and humidity which are highly conductive to rapid multiplication of pests (Kannan et al. 1992; Lakshmi 1993). However inappropriate application of pesticides affects the whole ecosystem, as their residues enter into the food chain and also pollute the soil, air, ground and surface water (ICAR 1967; UN/DESA 2008). More pesticide load in the environment may be enhanced due inability of soil microbial strains to breakdown them.

Humans are exposed to pesticides found in soil, water, air and food by different routes of exposure, such as inhalation, ingestion and dermal contact (Rekha and Naik 2006). Increasing incidence of human diseases, like cancer, chronic kidney diseases, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and behavioral disorders, especially among children, has been attributed to chronic pesticide poisoning (Agnihotri 1999). Pesticide pollution affects the lives of birds, wildlife, domestic animals, fishes, livestocks as well as the whole biodiversity (Anonymous 1991). Orgnochlorine insecticides (DDT, hexachlorocyclohexane, aldrin and dieldrin) are the most commonly used pesticides in the developing countries of Asia, because of their low cost and versatile use against various pests (Lallas 2001; Gupta 2004; FAO 2005). Pesticides, which are readily degraded in the environment, are called non-persistent insecticides (methoxychlor, sevin, carbaryl, malathion, lindane), herbicides (paraquat, dalapon, dacthal, treflan) and fungicides (benlate, mancozeb, zineb, captan). On the other hand, the pesticides, which are not readily degraded, are called persistent insecticides (DDT, aldrin, dieldrin, and chlordane), herbicides (simazine, turbacil, tordon), fungicides (PMAS, calo-clor, cadmium compounds). Furthermore, other pesticides, such as endosulfan and lindane, are currently in use throughout the world (EPA 2002) and their presence in air, water, and soil is a problem of great concern. Their amelioration in the environment has, therefore, become an important taste.

Microbial application in soil is one of the important components of biodegradation of agricultural pesticide. As the natural microorganisms are continuously exposed to pesticides, these microorganisms develop catabolic processes to remove the toxic compounds. Biological degradation by microorganisms (fungi, bacteria, actinomycetes and viruses) can effectively remove pesticides, especially organochlorines, organophosphates and carbamates used in agriculture from the both soil and water environment. Use of microbes is the most important strategy for removing the pesticides by enzymatic degradation as compared to non-enzymatic

processes (Porto et al. 2011). Thus, biological degradation, which involves the use of effective microorganisms for degrading the complex pesticides into simple inorganic chemicals, is self-driven process (Wood 2008). Moreover, this process is more effective, less hazardous, economically viable, versatile and environment-friendly (You and Liu 2004). The pesticide degrading microorganisms (PDMs) also have the plant growth promoting activities (viz. production of plant growth hormones, biological nitrogen fixation, phosphate solubilization, hydrogen cyanide, siderophore and antagonistic properties) to enhance the sustainable agriculture (Verma et al. 2014).

1.1 Microbial Degradation of Chemical Pesticides

Bioremediation is an important process for removal of pesticides from the soil by use of microbial strains. Bioremediation is being used for the breakdown of chemicals in soil, ground water, sludges, industrial waters and gases. It may be carried out in one or more ways described as biotransformation, bioaccumulation, bio-mineralization, biodegradation and cometabolism. Among microorganisms, especially bacteria have been isolated from different parts of the world with amazing property to degrade xenobiotic contaminants. These bacteria e.g. *Pseudomonas*, *Arthobacter*, *Ralstonia* and *Rhodococcus* (Park et al. 2003); *Bacillus*, *Planococcus* and *Acetobacter* (Shakoori et al. 2000) and *Alcaligenes* (Padmanabhan et al. 2003), have more potential for degrading pesticides (Table 1). Pollutants are also removed from the soil by endogenous microorganisms including bacteria with their extraordinary ability to use a wide variety of xenobiotics as sole energy and carbon source (Siddique et al. 2003). That is why microorganisms are termed as nature's biodegraders. They are scavengers in nature, responsible for recycling most of the natural waste materials into harmless compounds. Besides, microorganisms are highly adaptive and develop the capability to degrade recalcitrant compounds, particularly synthetic compounds through evolution of new genes, which encode enzymes that can use these compounds as their primary substrate (Suenaga et al. 2001). Many pesticide degradation genes, present in soil bacteria, have been shown to reside on plasmids, a common location for other degradation genes (Laemmli et al. 2000). Plasmids that bear genes encoding for enzymes capable of degradation, have been of great attraction in recent years. These plasmids provide organisms an ability to degrade certain compounds. Many catabolic plasmids have been found in species of *Pseudomonas, Alcaligenes, Actinobacter, Flavobacterium, Klebsiella, Moraxella* and *Arthrobacter* (Sayler et al. 1990). *Pseudomonas aeruginosa* MRM6, *Pseudomonas aeruginosa* SFM4 and *Acromobacter xylosoxidans* ES9 have been reported for the degradation of endosulfan which is widely used.

The survival of organism under pesticide stress can provide efficient, cheaper and environment friendly solution for bioremediation of xenobiotic-contaminated soil (Pallud et al. 2004). Microorganisms play an important role in the metabolism

Table 1 Pesticide degrading enzyme of various microbial strains and their co-factors

Enzyme	Cofactor requirements	Source organism(s)	Documented target pesticide(s)	Current bioremediation strategies employed
Oxidoreductases				
Gox	Flavin (FAD)	*Pseudomonas* sp. L Br; *Agrobacterium* strain T10	Glyphosate	In plant
Monooxygenases				
Esd	Flavin and NADH	*Mycobacterium* sp.	Endosulfan and Endosulfate	Not yet in use
Ese	Flavin (FMN)	*Arthrobacter* sp.	Endosulfan and Endosulfate	Not yet in use
Cyp1A1/1A2	Heme and NADH	Mammalian (Rat)	Atrazine, Norflurazon and Chlortoluron	In plant
Cyp76B	Heme and NADH	*Helianthus tuberosus*	Linuron, Chlortoluron and Isoproturon	In plant
P450	Heme and NADH	*Pseudomonas putida*	Hexachlorobenzene and Pentachlorobenzene	Transgenic *Sphingobium chlorophenolicum*
Dioxygenase				
TOD	Fe^{2+} and NADH	*Pseudomonas putida*	Trifluralin herbicides	Not yet in use against pesticides
E3	None	*Lucilia cuprina*	Synthetic pyrethroids and phosphotriester insecticides	Not yet in use
Phosphodiesterases				
PdeA	None	*Delftia acidovorans*	Organophosphorus compounds	Free-enzyme bioremediation
Phosphotriesterases				
OPH	Fe^{2+} and Zn^{2+}	*Agrobacterium radiobacter Pseudomonas diminuta*	Insecticides phosphotriester	Free-enzyme bioremediation
OpdA	Fe^{2+} and Zn^{2+}	*Flavobacterium* sp.	Parathion, methyl parathion, malathion, coumaphos, others	

(continued)

Table 1 (continued)

Enzyme	Cofactor requirements	Source organism(s)	Documented target pesticide(s)	Current bioremediation strategies employed
Phosphonatase				
Phn	None	*Escherichia coli Sinorhizobium meliloti*	Organophosphorus compounds	
Haloalkane				
Dehalogenase LinB	None	*Sphingobium* sp.; *Sphingomonas* sp.	Hexachlorocyclohexane (β—and δ—isomers)	Bioaugementation with *Sphingobium indicum*
AtzA	Fe^{2+}	*Pseudomonas* sp. ADP	Chloro-s-triazine herbicides	In plant and GM bacteria
TrzN	Zn^{2+}	*Nocardioides* sp.	Chloro-s-triazine herbicides	Not yet in use
LinA	None	*Sphingobium* sp.; *Shingomonas* sp.	Hexachlorocyclohexane (γ-isomer)	Bioaugementation with *Sphingobium indicum*
TfdA	α-ketoglutarate and Fe	*Ralstonia eutropha*	2,4-dichlorophenoxyacetic acid and pyridyl-oxyacetic	Not yet in use
DMO	NADH and a Rieske Fe-S centre	*Pseudomonas maltophilia*	Dicamba	Not yet in use
C-P-lyase				
Glp A&B	None	*Pseudomonas pseudomallei*	Organophosphorus compounds	Not yet in use
ND				
hocA	None	*Pseudomonas monteilli*	Organophosphorus compounds	Not yet in use
Mpd	None	*Pseudomonas*	Organophosphorus compounds	Not yet in use

Source Verma et al. (2014)

of organochlorine insecticides. Soil microorganisms are found capable of degrading several chlorinated pesticides by dehalogenation. DDT is slowly converted to DDE by *Aerobacter aerogenus*. The bacteria, like *Clostridium sporogenes* and *Bacillus coli*, produce trace amount of bengene and mono-chlorobenzene from lindane which can further be metabolized to produce CO_2 from sub-merged soils. Further, *Pseudomonas putida* and *Acinetobacter* play a crucial role in the dehalogenation of chloro-aromatic compounds (Vollmer et al. 1998). The oxidation reactions, which are important in degrading organochlorine insecticides in higher organism, are less common in microorganisms, probably due to the lack of a defined mixed-function oxidase system in microorganism. Thus, microorganisms are capable of carrying out a number of different oxidation reactions. Pesticides containing nitro substituents are reduced to amines. Many organisms can also reduce penta-chlorobengene to pentachloroaniline (Chacko et al. 1966). Many pesticides are degraded by amide or ester hydrolysis mediated by soil microorganisms. Matsumura and Boush (1966) isolated several microbes capable of degrading malathion through hydrolysis of the carboxylester group and demethylation. The cleavage of the aromatic rings is a common reaction, when soil microorganisms degrade pesticides. However, this process is one not usually encountered in higher animals. Thomas and Parkins (1995) have reported that atrazine degraders had two categories one degrades the side chains while other degrades the ring. Besides, some microorganism can condense or conjugate pesticides (Burchfied and Storrs 1957).

For minimization of soil pollution in the agricultural fields, indigenous microbial degradation is an efficient technology. Pesticide degradation is the breaking down of toxic pesticide into non-toxic compounds. However, sometimes intermediate compounds are formed which are also toxic for flora and fauna of soil and water environment. Thus, the most common type of degradation is carried out in the soil by microorganisms; especially the fungi and bacteria. Therefore, they are thought to play an important role in the removal and detoxification of these toxicants from the environment. Many bacteria, that are able to degrade carbamate pesticides, have been isolated from soil round the world (Desaint et al. 2000). Therefore, isolation of indigenous bacteria capable of metabolizing organophosphate compounds has received considerable attention, because these bacteria provide an environmental-friendly method of in situ detoxification of pesticides and other recalcitrant compounds.

2 Factors Effecting Degradation Process of Pesticides

Several degradative processes are involved in reducing the pesticide toxicity in soil. Many factors, such as physico-chemical properties of pesticides, characteristic of soil and environmental condition and management influence degradation process.

2.1 *Physico-Chemical Properties of Pesticides*

2.1.1 Structure of Pesticide

The physico-chemical property of pesticide and its degradation ability are determined by its chemical structure. Any type of change in its chemical structure causes a drastic change in susceptibility of compounds to biotransformation (Pal et al. 2006). Naumann (2000) reported that presence of halogen and chlorine drastically reduced biodegradation process, because a cage like structure formed by halogens reduced carbon source availability for bacteria. According to Cork and Krueger (1991) introduction of polar groups, such as OH, CHOOH and NH_2 in pesticide structure may provide the favourable condition for microbial system and also play major role in initiation of degradation of pesticide. Minor difference in the position or nature of pesticide of the same class can influence the rate of degradation (Topp et al. 1997). However, there is no clear relationship between the chemical properties of pesticides and their rates of degradation, as several phenomena are simultaneously involved in the degradation process of different pesticides (Calvet et al. 2005; Pal et al. 2006).

2.1.2 Pesticide Concentration

Concentration of pesticide plays an important role in determining the rate of biodegradation. Degradation kinetics of many pesticides shows invariably first order reaction i.e. the rate of degradation decreases with residual pesticide concentration (Pal et al. 2006). Prakash and Devi (2000) have reported that at higher initial concentrations of butachlor, the degradation rate was reduced due to limitation in the reaction sites in soil and toxic effect on microbial system or enzyme inhibition. Yu et al. (2003) reported that the half-life of butachlor in non-rhizosphere zone and inoculated rhizosphere soils of wheat ranged from (6.3 to 18.0) days at 1.0 mg kg^{-1}, (2.9 to 19.9) days at 10.0 mg kg^{-1} and (10.8 to 23.2) days at 100.0 mg kg^{-1}. This indicates that the degradation of butachlor depends on application rate of pesticide and soil type.

2.1.3 Pesticide Solubility

Solubility of pesticide in soil is influenced by temperature, pH, polarity of the substance, hydrogen bonding, molecular size and the method used (Zacharia 2011). Solubility of pesticide plays a significance role in the degradation of pesticide, because low water solubility makes it more resistant to microbial degradation than higher water solubility. Microorganism can obviously use only the dissolved fraction of the pesticide in the solution. Therefore, the rate of biodegradation of pesticide largely depends up on the rate of its dissolution in the water (Cork and Krueger 1991).

2.2 *Effect of Soil Structure on the Degradation of Pesticides in Soils*

2.2.1 Soil Structure

Soil plays a critical role in pesticide biodegradation, because it provides a favourable environment for the degradative microorganism. Soil properties (clay content, organic matter and pH etc.) greatly affect the rate of degradation of pesticide in soil, because its physio-chemical properties govern the sorption bioavailability abd persistence of pesticide in the soil (Pal et al. 2006; Zacharia 2011). Gold et al. (1996) reported that soil pH and clay content greatly affected the persistence of pesticide (chlropyriphos, cypermethrin, permethrin and fenvelerate etc.) under the field condition.

2.2.2 Soil Moisture

Water acts as a solvent for the pesticide movement and diffusion and is also essential for microbial function. The rate of degradation of pesticide increases with water content, because the rate of diffusion of atmospheric oxygen into soil is limited. In this situation and anaerobic pesticide transformation can prevail over the aerobic degradation (Skopp et al. 1990). However, poor oxygen transfer in a high water content soil can retard or accelerate the degradation of pesticide. Walter-Echols and Lichtenstein (1978) reported that phorate was more persistent in flooded field than non-flooded. Schroll et al. (2006) showed an optimum mineralization for isoproturon, benazolin-ethyl, and glyphosate at a soil water potential of 0.015 MPa, whereas pesticide mineralization was considerably reduced when soil moisture approximated water holding capacity. Soil moisture affects not only the microbic activity but also diversity of soil microbes (Bouseba et al. 2009). The reduction of the microbial activity at lower soil water content could increase the contact times between the pesticide and the soil components, and so favour long-term sorption (Cox and Walker 1999).

2.2.3 Temperature

Effect of temperature on rate of pesticide degradation largely depends on molecular structure of pesticide. Temperature affects the adsorption by altering the solubility and hydrolysis of pesticide in soil (Burns 1975; Racke et al. 1997). Ismail et al. (2012) found that half-life of cypermethrin decreased from 5.46–3.25 week and 4.25–2.98 weeks in the peat soil and silty clay, respectively when the soil temperature was increased from 25–35 °C (dark). Rani and Sud (2015) reported that pesticide had a potential to contaminate surface and ground water at higher temperature due to weak adsorption on tested soils and release of more adsorbed

pesticide during desorption with water. At temperature below 20 °C, pesticide becomes almost immobile and therefore, soil remediation may be required.

2.2.4 Soil pH

Soil pH plays an important role in both abiotic/biotic degradation and also in the adsorption of ionic pesticide (Chaplain et al. 2011; Pal et al. 2006). Most pesticide formulations, such as dry flowables, emulsifiable concentrates and wettable powders are designed to be diluted with water as the carrier. A water pH higher than 7, which creates alkaline conditions, can cause some pesticides to undergo degradation or chemical breakdown, a process known as hydrolysis. In general, insecticides are more susceptible to hydrolysis than are fungicides, herbicides, defoliants or growth regulators. Usually, organophosphate and carbamate insecticides are more susceptible than chlorinated hydrocarbon insecticides. Some pyrethroids also exhibit susceptibility to hydrolysis (Deer and Beard 2001). Thus, soil pH greatly influences the sportive behaviour of pesticide molecules on clay and organic surface.

2.2.5 Soil Organic Matter

Behaviour of pesticide in soils can change in the presence of the organic matter. Soil organic matter either enhances the microbial activity by co-metabolism or decreases the microbe-mediated pesticide degradation by stimulating adsorption process (Thom et al. 1997; Perucci et al. 2000; Pal et al. 2006). Burns (1975) found that a minimum level of organic matter (>1.0 %) is essential to ensure the presence of an active autochthonous microbial population which can degrade pesticides.

3 Enzymes Involved in Degradation Process

Pesticide degradation is a process that involves the complete rupture of an organic compound into inorganic constituents of pesticide by degrading microbes. The biodegradation involves transferring the substrates and products within a well-coordinated microbial community. This process is referred as metabolic co-operation under natural environments (Abraham et al. 2002). Enzyme-based pesticide degradation process is an innovative treatment technique for removal of pesticide from the polluted environments. Fungi and bacteria are considered as the extracellular enzyme producing microorganisms for the degradation of pollutant. White rot fungi have been used as promising bioremediation agents, especially for compounds not easily degraded by bacteria. The production and secretion of extracellular enzymes by microbes due environmental impact are very interesting. Some of these extracellular enzymes are involved in lignin degradation, such as

lignin peroxidase, manganese peroxidase, laccase and oxidases. Several microbial strains that degrade pesticide have been listed in the Table 1. Transferases, isomerases, hydrolases and ligases are extracellular enzymes responsible for the biodegradation of the pesticides. These enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage etc.

3.1 Oxidoreductases

Oxidoreductase is an enzyme that catalyses both oxidation and reduction reactions. It is an important enzyme, like glyphosate oxidase (GOX), for glyphosate degradation. This enzyme is a flavoprotein amine oxidase isolated from *Pseudomonas* sp. LBr which catalyses the oxidation of glyphosate to form aminomethylphosphonate (AMPA) and releases the keto acid glyoxylate (Scott et al. 2008). Similarly, *Mycobacterium tuberculosis* ESD strains secrete endosulfan monooxygenase II enzyme which degrades the β-endosulfan to monoaldehyde and hydroxyether, but transforms alpha-endosulfan to the more toxic endosulfan sulfate. Alternatively, hydrolysis of endosulfan in some bacteria (*Pseudomonas aeruginosa*, *Burkholderia cepacia*) yields the less toxic metabolite i.e. endosulfan diol (Kumar et al. 2007). Endosulfan can get spontaneously hydrolyzed to the diol in the alkaline conditions. *Phanerochaete chrysosporium* and *T. versicolor* have the ability to degrade simazin, dieldrin and trifluralin pesticides independently by their laccase activity (Fragoeiro and Magan 2005).

3.2 Monooxygenases

Monooxygenases generally catalyse the transfer of one atom of O_2 to an organic compound and the other gets reduced by electrons from co-factors to yield water. Monooxygenases often play a role in the metabolism of xenobiotics by increasing either their reactivity and/or the water solubility through the addition of an oxygen atom.

3.3 Cytochrome P450 Oxidoreductases

The cytochrome P450 family is a large and well characterised group of monooxygenase enzymes. They have been long recognised for their potential in many industrial processes, particularly due to their ability to oxidise or hydroxylate

substrates using molecular oxygen (Scott et al. 2008). Cytochrome P450 oxidoreductases helps in degradation of atrazine, norflurazon and chlortoluron from soils. Cytochrome CYP76B1 isolated from *Helianthus tuberosus* was cloned into tobacco and *Arabidopsis* sp. This enzyme has capability to catalyse the oxidative dealkylation of phenylurea herbicides, such as linuron, chlortoluron and isoproturon (Didierjean et al. 2002). Prokaryotic cytochrome P450s isolated from *Pseudomonas putida* (Chen et al. 2002) and *Sphingobium chlorophenolicum* have shown great potential to degrade chlorinated pentachlorobenzene and hexachlorobenzene (Yan et al. 2006).

3.4 Dioxygenases

Ring hydroxylating dioxygenases (RHDOs) are one of the most important classes of enzymes featuring in the microbial metabolism of several aromatic compounds Toluene dioxygenases (TOD) enzyme, isolated from *P. putida* F1, is highly used for the degradation of toluene, polychlorinated hydrocarbons, ethylbenzene and *p*-xylene.

3.5 Hydrolases

Hydrolases are a broad group of enzymes which help in the pesticide biodegradation. They catalyze the hydrolysis of several major biochemical classes of pesticides (esters, peptide bonds, carbon-halide bonds, ureas, thioesters, etc.) (Scott et al. 2008). Organophosphate hydrolases (OPH) or Phosphotriesterases were isolated from *Pseudomonas diminuta* and *Flavobacterium* sp. The first isolated phosphotriesterase from *Pseudomonas diminuta* strain MG was encoded by a gene called *opd* (organophosphate-degrading). This showed a highly catalytic activity towards organophosphate pesticides. These enzymes specifically hydrolyze phosphoester bonds, such as P–O, P–F, P–NC, and P–S (Ortiz-Hernández et al. 2003). Other microbial enzymes, such as organophosphorus hydrolase (OPH; encoded by the *opd* gene), methyl-parathion hydrolase (MPH; encoded by the *mpd* gene), and hydrolysis of coroxon (HOCA; encoded by the *hocA* gene), were isolated from *Flavobacterium* sp. (Sethunathan and Yoshida 1973), *Plesimonas* sp. strain M6 (Cui et al. 2001) and *Pseudomonas moteilli* (Horne et al. 2002), respectively.

3.6 Esterase

Esterases are enzymes that catalyze hydrolysis reactions for carboxylic esters (carboxiesterases), amides (amidases), phosphate esters (phosphatases) (Ortiz-Hernández et al. 2013). Esterases have been cloned and their proteins have

been sequenced from several microorganisms, e.g. two ferulic acid esterases from *Aspergillus tubingensis*, a cephalosporin esterase from the yeast *Rhodosporidium toruloides*, a chrysanthemic acid esterase from *Arthrobacter globiformis*, and several other esterases from *Pseudomonas fluorescens* strain. These enzymes are used for the biodegradation of various types of organophosphate, and other herbicides and insecticides.

3.7 Phosphotriesterases

The phosphotriesterase enzyme is a homo-dimeric protein with a monomeric molecular weight of 36 Kda. This enzyme has potential use for the cleaning of organophosphorus pesticides-contaminated environments (Ortiz-Hernández et al. 2003). The first isolated phosphotriesterase from *Pseudomonas diminuta* MG showed high catalytic activity towards organophosphate pesticides (Ortiz-Hernández et al. 2013). This enzyme specifically hydrolyzes phosphoester bonds, such as P–O, P–F, P–NC, and P–S, and the hydrolysis mechanism involves a water molecule at the phosphorus centre (Ortiz-Hernández et al. 2003).

3.8 Dehalogenases

Bacterial dehalogenasescatalyze the cleavage of halogen substituents in haloaromatics, haloalkanes, haloalcohols, and haloalkanoic acids (Fetzner and Lingens 1994). Haloalkane dehalogenases (Dh1A) enzyme was encoded by gene *LinB* isolated from *Xanthomonas thobacter autotrophicus* GJ10; haloacetate dehalogenase (DehH1) from *Moraxella* sp.; and 2-hydroxymuconic semialdehyde hydrolase (DmpD) from *Pseudomonas* sp. CF600 which are responsible for degradation of hexachlorocyclohexane (HCN).

4 Mechanism and Pathways of Degradation

Pesticides are degraded by many different mechanisms. Physical, chemical and biological methods play an important role in the transformation of pesticides (insecticides, herbicides and fungicides) into their degrading products (Coats 1991). The metabolism of pesticides involves three phase processes; in first phase process, the initial properties of a parent compounds are transformed through oxidation, reduction, hydration or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent compound, in second phase, process involves conjugation of a pesticide to a sugar or amino acid, which increases the water solubility and reduces toxicity compared to the parent pesticide, and in third

phase process, helps in conversion of second phase metabolites into secondary conjugates, which are also non-toxic. In these processes, fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc. (Van Herwijnen et al. 2003; Ortiz-Hernández et al. 2011).

4.1 Physical Degradation

Light and heat are two primary physical agents involved in the degradation process of pesticides. Photolysis of pesticide residues is extremely significant on vegetation, soil surface, water and also in the atmosphere. Photodegradative reactions play an important role in thermal decomposition of the organic compounds. Low temperature, especially freezing, can also contribute occasionally to pesticide degradation, if certain formulations are allowed to freeze, and the pesticide is forced out of solution, suspension, or microencapsulation, making it more susceptible to degradative forces before and after application (Coats 1991).

4.2 Chemical Degradation

Presence of reactive agents in the formulation of pesticide and its application in the environment are responsible for chemical degradation. Water is the main source for substantial breakdown of pesticide in solution, particularly in conjunction with pH extremes. Variance in neutral pH can stimulate rapid decomposition of pH-sensitive compounds. It was mostly observed that oxygen deficit environments caused oxidative transformations for pesticide degradation. Several reactive forms of molecular oxygen, such as ozone, superoxide and peroxide, are capable of reacting with many chemicals to generate oxidative products. Some inorganic redox reagents can also play an important role as highly effective catalysts which cause to pesticide transformation in soils and aquatic environment (Coats 1991).

4.3 Biological Degradation

Microorganism (bacteria, fungi and actinomycetes) are the most important group of degraders, based on their common occurrence in soil and water (Bollag 1974; Alexander 1981). Three major degradation strategies are exhibited by microbes:

(i) Co-metabolism is the biotransformation of a pesticide molecule co-incidental to the normal metabolic functions of the microbial life processes (growth, reproduction, dispersal) (Coats 1991).

(ii) Catabolism is the utilization of an organic molecule as a nutrient or energy source. Some pesticides have been observed to be susceptible to enhanced microbial degradation (EMD) by populations of microbes (principally bacteria) that have adapted, following repeated use, to utilize the pesticide molecule as a sole source of carbon or nitrogen (Racke and Coats 1990; Coats 1991).

(iii) Microorganisms secrete enzymes into the soil for digestion of substrates. The enzymes, e.g. phosphatases and amidases, may persist in the soil for long after the parent microbial cell is dead. The stability and persistence of these extracellular enzymes can provide soil with various important biochemical catalytic capabilities (Racke and Coats 1990; Coats 1991).

4.4 Metabolic Pathway of Degradation

The metabolism of pesticides involves three phase process; in first phase process, the initial properties of a parent compound are transformed through oxidation, reduction, hydration or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent, in the second phase, a process involves conjugation of a pesticide to a sugar or amino acid, which increases the water solubility and reduces toxicity compared to the parent pesticide, and in the third phase, a process helps in conversion of second phase metabolites into secondary conjugates, which are also non-toxic as shown in Table 2 (Van Herwijnen et al. 2003; Ortiz-Hernández et al. 2011).

Table 2 Three phases of pesticide metabolism

Characteristics	Initial properties	Phase I	Phase II	Phase III
Reactions	Parent compound	Oxidation, hydrolysis, reduction	Conjugation	Secondary conjugation or incorporation into biopolymers
Solubility	Lipophilic	Amphophilic	Hydrophilic	Hydrophilic or insoluble
Phytotoxicity	Toxic	Modified or less toxic	Greatly reduced or nontoxic	Nontoxic
Mobility	Selective	Modified or reduced	Limited or immobile	Immobile or unavailable
Bioavailability	Readily absorbed in GI tract of animals	Readily absorbed in GI tract of animals	Less absorption	Limited absorption

5 Microbial Toxicity of Pesticides and Degradation Products

Toxicity of pesticides is investigated during registration process, but the toxicity of degradation products to the soil microorganisms is still unexplored. Pesticides interact with metabolic activities and also physiological and biochemical behavior of soil microbes. Many recent studies reveal the adverse impact of pesticide on soil microbial biomass and soil respiration. Wardle and Parkinson (1990) found that microbial variables only occurred at herbicide (2, 4-D, picloram and glyphosate) concentrations much higher than field application. However, the side-effects of these chemicals are not much of ecological significance. Zhang et al. (2014) reported that tetraconazole fungicide decreased the soil microbial biomass and activity, and also changed the structures of the soil microbial community. Tejada et al. (2015) reported that cypermethrin insecticide caused a negative effect on soil enzymatic activities and microbial diversity. This negative impact was greater when a high dose of insecticide was used. This impact was also enhanced in the soil with lower organic matter content. A detailed description of toxicity of various pesticides on soil microbial communities is summarized in Table 3. Generally, long-term application of pesticides can disturb microbial diversity and its enzyme activity, which can reduce number of microorganism and soil fertility. Therefore, understanding the mechanisms of molecular responses to microbes could be helpful in elucidating the risk assessment of pesticide contaminations and its consequent adverse impacts on soil microbial diversity, enzymatic activities, and soil fertility (Khare and Arora 2015).

6 Challenges of Microbial Degradation of Pesticides

The main problem of popularization of microbial consortia for the degradation of pesticides in soil is the unavailability of the indigenous and effective microbial consortia in the market for the farmers use (Verma et al. 2014). The specific microbial strain for degradation of specific pesticide is also not available in the market for the farmers, as all microbial strains do not degrade all type of pesticides. Due to these difficulties, biopesticide may serve as only alternative for the pest management in agro-ecosystem. The use of genetic engineering tool and techniques to form genetically modified microorganism for the rapid degradation of pesticides in different environments is also very promising which will facilitate the production of genetically modified bio-pesticide for the prevention of pest attack to the agricultural crops. Another important aspect is that the effective pesticide degrading microorganisms and biopesticide preparations are very sensitive to heat, desiccation and ultraviolet radiations which reduce the effectiveness of the bio-pesticides. Special formulations of bio-pesticide and pesticide degrading microbial consortia in non-toxic carrier material are sought to be available for the long term storage at normal temperature.

Table 3 Toxicity of pesticide on soil microbes

Pesticide	Type	Toxicity on soil microbes	Reference
Cypermethrin	Insecticide	Caused a negative effect on soil enzymatic activities and microbial diversity	Tejada et al. (2015)
Tetraconazole	Fungicide	Decreases the soil microbial biomass and activity and changes the structures of the soil microbial community	Zhang et al. (2014)
Imidacloprid	Insecticide	Insecticide induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities	Cycon et al. (2013)
Glyphosate	Herbicide	Studies suggest that the changes in the dissipation or distribution of glyphosate following repeated applications of glyphosate may be related to shifts in the soil microbial community composition	Lancaster et al. (2010)
Metam sodium	Fungicide	Significantly altered the structure of the soil microbial community with the former having a more prominent effect	Spyrou et al. (2009)
Methamidophos	Insecticide	Decrease in species richness, changes in bacterial community structure	Wang et al. (2008)
Atrazine, Isoproturon, Metribuzin, Sulfosulfuron	Herbicicde	Adversely affected *Bradyrhizobium* sp	Khan et al. (2006)
Methamidophos	Insecticide	Decreased microbial biomass (41–83 %)	Wang et al. (2006)
Diazinon, Imidacloprid	Insecticide	Inhibited the growth of Proteus vulgaris, a urease-producing bacterium	Ingram et al. (2005)
Carbendazim, Imazetapir, Thiram	Herbicide and Insecticide	Inhibited nodulation; reduced nitrogenase activity in rhizobia	Niewiadomska (2004)
Mefenoxam, metalaxyl	Fungicide	Inhibited N-fixing bacteria	Monkiedje et al. (2002)
Bensulfuron methyl, metsulfuron methyl	Herbicide	Decreased microbial biomass-C, and N	El-Ghamry et al. (2001)
Captan, Apron, Arrest and Crown	Fungicide	Except crown, all fungicides decreased viable counts of rhizobia	Kyei-Boahen et al. (2001)
Metalaxyl	Fungicide	Decreased microbial biomass	Sukul and Spiteller (2001)
Carbofuran, Ethion	Insecticide	Adversely affected soil microorganisms	Kalam and Mukherjee (2001)
Hexaconazole	Herbicide	Reduction in nitrogenase activity of *Anabaena doliolum*	Hammouda (1999)

(continued)

Table 3 (continued)

Pesticide	Type	Toxicity on soil microbes	Reference
2,4-D, Picloram, Glyphosate	Herbicides	Microbial variables only occurred at herbicide (2, 4-D, picloram and glyphosate) concentrations of much higher than that which occurs following field application, the side-effects of these chemicals is probably of little ecological significance	Wardle and Parkinson (1990)
Lindane	Insecticide	Nitrification and phosphate solubilization decreased	Ogunseitan and Odeyemi (1985)

7 Future Prospectives

Recent approaches, like integrated nutrient management and integrated pest management, are necessary to increase sustainable agricultural productivity and conservation of natural resources. Agricultural development continues to remain the most important sector of Indian planning and policy. In the process of development of agriculture, pesticides are important tools as a plant protection agent for boosting food production. No doubt, pesticides have been used indiscriminately resulting in sever environmental contamination. In order to minimize the toxic effect of pesticides in soil and water, we have to use potential microbes to degrade pesticides effectively as residues. The microorganisms, which utilize pesticides for obtaining energy, could be the best candidates for bioremediation of pesticides-contaminated soil or water. Biodegradation is a cheaper, more effective and natural way to combat the residual problem of man-made chemicals. These isolates have the potential to clean up the environment from pesticide pollutant. These pesticide-degrading microorganism also have ability to promote plant growth and yield due to production of phytohormones, ammonia, siderophore and biological nitrogen fixation, phosphate solubilization and also antagonistic activity against phytopathogenic microorganism. The pesticide degrading microorganism also improves the soil health and fertility as well as enhances sustainable agriculture.

8 Conclusion

In current scenario, farmers are using excessive pesticides and chemical fertilizers in unmanaged manner for higher production of grain yield. So, various pesticides and chemical fertilizers invariably cause the pollution of water, soil and air, and also many human diseases. It is also causes loss of soil fertility and soil health. More industries of pesticides and chemical fertilizers are discharging their toxic products in waste water which create the environmental problem. The effective microbial

consortium of pesticide degrading microorganism could be used for degradation of pesticides effectively in both soil and water. Investigation of biodegradation by microorganisms in the natural environment has revealed a larger number of enzymatic reactions with high bioremediation potential. These biocatalysts may be formed in large quantities by genetic engineering technology, expression of enzymes, or indigenous organisms, which are used in the agriculture field for removing pesticides from polluted areas. The effective and indigenous microbial consortia contribute significantly for the removal of toxic pesticides used in agriculture. These pesticides degrading microbial consortium may also serve as effective biofertilizers to substitute approximate 20–30 % chemical fertilizer. It also minimizes the chemical pesticide uses in agriculture, because microbial consortium will degrade the pesticide pollutant in simple form of chemical compound which is used by the crop plant. Conclusively, it is also economically viable and enhances the quality of life for farmers and society as a whole.

Acknowledgments Authors thankful to SERB (Science and Engineering Research Board), New Delhi, India for providing fund for project entitled “Studies of agriculturally important microorganism to develop effective microbial consortium for degradation of pesticide and insecticide in soil to enhance sustainable agriculture” to carry out research on pesticide degradation.

References

Abraham, W. R., Nogales, B., Golyshin, P. N., Pieper, D. H., & Timmis, K. N. (2002). Polychlorinated biphenyl-degrading microbial communities and sediments. *Current Opinion in Microbiology, 5*, 246–253.

Agnihotri, N. P. (1999). *Pesticide safety and monitoring*. New Delhi: All India Coordinated Research Project on Pesticides Residues, Indian Council of Agricultural Research.

Alexander, M. (1981). Biodegradation of chemicals of environmental concern. *Science, 211*, 132–138.

Anonymous. (1991). *Survey of the Environment*, The Hindu, Government of India, Eleventh Five-Year Plan (2008–2012) Planning Commission of India, New Delhi, http://planningcommission.nic.in/plans/planrel/fiveyr/welcome.html

Bhatnagar, V. K. (2001). Pesticides pollution: trends and perspectives. *ICMR Bulletin, 31*, 87–88.

Bollag, J. M. (1974). Microbial transformation of pesticides. *Advances in Applied Microbiology, 18*, 75–130.

Bouseba, B., Zertal, A., Beguet, J., Rouard, N., Devers, M., Martin, C., et al. (2009). Evidence for 2, 4-D mineralisation in mediterranean soils: Impact of moisture content and temperature. *Pest Management Science, 65*, 1021–1029.

Burchfield, H. P., & Storrs, E. E. (1957). Effect of chlorine substitution and isomerism on intractions of S-triazine derivatives with conidia of *Neurospora sitophilia*. *Boyce Thompson Institute for Plant Research, 18*, 429–452.

Burns, R. G. (1975). Factors affecting pesticide loss from soil. In E. A. Paul & A. D. McLaren (Eds.), *Soil biochemistry* (pp. 103–141). New York: Marcel Dekker Inc.

Calvet, R., Barriuso, E., Bedos, C., Benoit, P., Charnay, M. P., & Coquet, Y. (2005). *Les pesticides dans les sols*. Conséquences agronomiqueset environnementales (Editions France Agricole), Dunod, ISBN 2-85557-119-7, Paris.

Chacko, C. I., Lockwood, J. L., & Zabik, M. (1966). Chlorinated hydrocarbon pesticides: Degradation by microbes. *Science, 154*, 893–895.

Chaplain, V., Mougin, C., Barriuso, E., Mamy, L., Vieublé-Gonod, L., & Benoit, P., et al. (2011). *Fate of pesticides in soils: Toward an integrated approach of influential factors*. INTECH Open Access Publisher.
Chen, X., Christopher, A., Jones, J. P., Bell, S. G., Guo, Q., Xu, F., et al. (2002). Crystal structure of the F87W/Y96F/V247L mutant of cytochrome P-450 cam with 1, 3,5 trichlorobenzene bound and further protein engineering for the oxidation of pentachlorobenzene and hexachlorobenezene. *Journal of Biological Chemistry, 277*, 37519–37526.
Coats, J. R. (1991). Pesticide degradation mechanisms and environmental activation. In *ACS Symposium Series-American Chemical Society (USA)*.
Cork, D. J., & Krueger, J. P. (1991). Microbial transformation of herbicide and pesticides. *Advances in Applied Microbiology, 36*, 1–66.
Cox, L., & Walker, A. (1999). Studies of time-dependent sorption of linuron and isoproturon in soils. *Chemosphere, 38*(12), 2707–2718.
Cui, Z., Li, S., & Fu, G. (2001). Isolation of methyl-parathion-degrading strain M6 and cloning of the methyl-parathion hydrolase gene. *Applied and Environmental Microbiology, 67*, 4922–4925.
Cycon, M., Markowicz, A., Borymski, S., Wojcik, M., & Piotrowska-Seget, Z. (2013). Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. *Journal of Environmental Management, 131*, 55–65.
Deer, H. M., & Beard, R. (2001). Effect of water pH on the chemical stability of pesticides. *AG/Pesticides, 14*, 1.
Desaint, S., Hartmann, A., Parekh, N. R., & Fournier, J. C. (2000). Genetic diversity of carbofuran-degrading soil bacteria. *FEMS Microbiology Ecology, 34*, 173–180.
Didierjean, L., Gondet, L., Perkins, R., Lau, S. M. C., Schaller, H., O'Keefe, D. P., et al. (2002). Engineering herbicide metabolism in tobacco and Arabidopsis with CYP76B1, a cytochrome P450 enzyme from Jerusalem artichoke. *Plant Physiology, 130*(1), 179–189.
El-Ghamry, A. M., Huang, C. Y., & Xu, J. M. (2001). Combined effects of two sulfonylurea herbicides on soil microbial biomass and N-mineralization. *Journal of Environmental Sciences, 13*, 311–317.
EPA. (2002). *EPA's National Service Center for Environmental Publications*. Cincinnati: Endosulfan RED Facts. http://www.epa.gov/pesticides/reregistration/endosulfan/S
FAO. (2005). *Proceedings of the Asia Regional Workshop*. Bangkok: Regional Office for Asia and the Pacific.
Fetzner, S. R., & Lingens, F. (1994). Bacterial dehalogenases. *Microbiological Reviews, 58*(4), 641–685.
Finley, S. D., Broadbelt, L. J., & Hatzimanikatis, V. (2010). Insilico feasibility of novel biodegradation pathways for 1, 2, 4-trichlorobenzene. *BMC Systems Biology, 4*, 4–14.
Fragoeiro, S., & Magan, N. (2005). Impact of hydrolytic enzyme activity of two white rot fungi on degradation of a mixture of three pesticides under osmotic stress. *Environmental Microbiology, 7*, 348–355.
Gold, R. E., Howell, H. N., Pawson, B. M., Wright, M. S., & Lutz, J. L. (1996). Persistance and bioavailability of termicides to subterranean termite from five soil types and location in Texas. *Sociobiology, 28*, 337–363.
Gupta, P. K. (2004). Pesticide exposure—Indian scene. *Toxicology, 198*(1), 83–90.
Hammouda, O. (1999). Response of the paddy field cyanobacterium Anabaena doliolum to carbofuran. *Ecotoxicology and Environmental Safety, 44*(2), 215–219.
Horne, I., Sutherland, T. D., Oakeshott, J. G., & Russell, R. J. (2002). Cloning and expression of the phosphotriesterase gene *hoc*A from *Pseudomonas monteilii* C11. *Microbiology, 148*, 2687–2695.
ICAR. (1967). *Report of the special committee on harmful effects of pesticides* (p. 78). ICAR: New Delhi.
Ingram, C. W., Coyne, M. S., & Williams, D. W. (2005). Effects of commercial diazinon and imidacloprid on microbial urease activity in soil and sod. *Journal of Environmental Quality, 34* (5), 1573–1580.

Ismail, B. S., Mazlinda, M., & Zuriati, Z. (2012). Effects of temperature, soil moisture content and soil type on the degradation of cypermethrin in two types of Malaysian agricultural soils. *World Applied Sciences Journal, 17*, 428–432.

Jain, R. K., Kapur, M., Labana, S., Lal, B., Sarma, P. M., Bhattacharya, D., et al. (2005). Microbial diversity: Application of microorganisms for the biodegradation of xenobiotics. *Current Science, 89*, 101–112.

Kalam, A., & Mukherjee, A. K. (2001). Influence of hexaconazole, carbofuran and ethion on soil microflora and dehydrogenase activities in soil and intact cell. *Indian Journal of Experimental Biology, 39*(1), 90–94.

Kannan, K., Tanabe, S., Ramesh, A., Subramanian, A., & Tatsukawa, R. (1992). Persistent orgnochlorine residues in food stuffs from India and their implications on human dietary exposure. *Journal of Agricultural and Food Chemistry, 40*, 518–524.

Khan, M. S., Chaudhry, P., Wani, P. A., & Zaidi, A. (2006). Biotoxic effects of the herbicides on growth, seed yield, and grain protein of green gram. *Journal of Applied Sciences and Environmental Management, 10*(3), 141–146.

Khare, E., & Arora, N. K. (2015). Effects of soil environment on field efficacy of microbial inoculants. In *Plant Microbes Symbiosis: Appl. Facet.* (pp. 353–381). India: Springer.

Kumar, K., Devi, S. S., Krishnamurthi, K., Kanade, G. S., & Chakrabarti, T. (2007). Enrichment and isolation of endosulfan degrading and detoxifying bacteria. *Chemosphere, 68*(2), 317–322.

Kyei-Boahen, S., Slinkard, A. E., & Walley, F. L. (2001). Rhizobial survival and nodulation of chickpea as influenced by fungicide seed treatment. *Canadian Journal of Microbiology, 47*, 585–589.

Laemmli, C. M., Leveau, J. H. J., Zehnder, A. J. B., & Van der Meer, J. R. (2000). Characterization of a second *tfd* gene cluster for chlorophenol and chlorocatechol metabolism on plasmid pJP4 in *Ralstonia eutropha* JMP134 (pJP4). *Journal of Bacteriology, 182*, 4165–4172.

Lakshmi, A. (1993). Pesticides in India: Risk assessment to aquatic ecosystems. *Science of the Total Environment, 134*, 243–253.

Lallas, P. L. (2001). The Stockholm Convention on persistent organic pollutants. *American Journal of International Law*, 692–708.

Lancaster, S. H., Hollister, E. B., Senseman, S. A., & Gentry, T. J. (2010). Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate. *Pest Management Science, 66*, 59–64.

Matsumura, F., & Boush, G. M. (1966). Malathion degradation by *Trichoderma viride* and a *Pseudomonas* species. *Science, 153*, 1278–1280.

Monkiedje, A., Ilori, M. O., & Spiteller, M. (2002). Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. *Soil Biology & Biochemistry, 34*, 1939–1948.

Naumann, K. (2000). Influence of chlorine substituents on biological activity of chemicals: A review. *Pest Management Science, 56*(1), 3–21.

Niewiadomska, A. (2004). Effect of carbendazim, imazetapir and thiram on nitrogenase activity, the number of microorganisms in soil and yield of red clover (*Trifolium pratense* L.). *Polish Journal of Environmental Studies, 13*(4), 403–410.

Ogunseitan, O. A., & Odeyemi, O. (1985). Effects of lindane, captan and malathion on nitrification, sulphur oxidation, phosphate solubilization, and respiration in a tropical soil. *Environmental Pollution, 37*(1), 343–354.

Ortiz-Hernández, M. L., Quintero-Ramírez, R., Nava-Ocampo, A. A., & Bello-Ramírez, A. M. (2003). Study of the mechanism of *Flavobacterium* sp for hydrolyzing organophosphate pesticides. *Fundamental & Clinical Pharmacology, 17*(6), 717–723.

Ortiz-Hernández, M. L., Sánchez-Salinas, E., Dantán-González, E., Castrejón-Godínez, M. L. (2013). Pesticide biodegradation: Mechanisms, genetics and strategies to enhance the process. In R. Chamy, F. Rosenkranz (Eds.), *Biodegradation-life of science* (pp. 251–287). Intech.

Ortiz-Hernández, M. L., Sánchez-Salinas, E., Olvera-Velona, A., Folch-Mallol, J. L. (2011). Pesticides in the environment: Impacts and its biodegradation as a strategy for residues treatment. In M. Stoytcheva (Ed.), *Pesticides-formulations, effects, fate, In-Tech*, doi:10.5772/13534. Available from: http://www.intechopen.com/books/pesticides-formulations-effects-fate/pesticides-in-the-environment-impacts-and-itsbiodegradation-as-a-strategy-for-residues-treatment

Padmanabhan, P., Padmanabhan, S., DeRito, C., Gray, A., Gannon, D., Snape, J. R., et al. (2003). Respiration of 13C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of 13C-labeled soil DNA. *Applied and Environmental Microbiology, 69*(3), 1614–1622.

Pal, R., Chakrabarti, K., Chakraborty, A., & Chowdhury, A. (2006). Degradation and effects of pesticides on soil microbiological parameters-A review. *International Journal of Agricultural Research, 1*(33), 240–258.

Pallud, C., Dechesne, A., Gaudet, J. P., Debouzia, D., & Grundmann, G. L. (2004). Modification of spatial distribution of 2,4-dichlorophenoxy acetic acid degrader microhabitats during growth in soil columns. *Applied and Environmental Microbiology, 70*, 2709–2716.

Park, J. H., Feng, Y., Ji, P., Voice, T. C., & Boyd, S. A. (2003). Assessment of bioavailability of soil-sorbed atrazine. *Applied and Environmental Microbiology, 69*, 3288–3298.

Perucci, P., Dumontet, S., Bufo, S. A., Mazzatura, A., & Casucci, C. (2000). Effects of organic amendment and herbicide treatment on soil microbial biomass. *Biology and Fertility of Soils, 32*, 17–23.

Porto, A. L. M., Melgar, G. Z., Kasemodel, M. C., Nitschke, M. (2011). Pesticides in modern world-pesticides use and management. In M. Stoytcheva (Ed.), *Biodegradation of pesticides*, Chapter 20, p 407. doi:10.5772/17686

Prakash, N. B., & Devi, L. S. (2000). Persistence of butachlor in soils under different moisture regime. *Journal of the Indian Society of Soil Science, 48*, 249–256.

Racke, K. D., Coats, J. R. (1990). Enhanced biodegradation of pesticides in the environment. In *ACS Symposium Series (No. 426)* (pp 53–67). American Chemical Society.

Racke, K. D., Skidmore, M. W., Hamilton, D. J., Unsworth, J. B., Miyamoto, J., & Cohen, S. Z. (1997). Pesticide fate in tropical soil. *Pure and Applied Chemistry, 69*, 1349–1371.

Rani, S., & Sud, D. (2015). Effect of temperature on adsorption-desorption behaviour of triazophos in Indian soils. *Plant Soil Environment, 61*(1), 36–42.

Rekha, S. N., & Naik, R. P. (2006). Pesticide residue in organic and conventional food-risk analysis. *Journal of Chemical Health and Safety, 13*, 12–19.

Sayler, G. S., Hooper, S. W., Layton, A. C., & King, J. M. H. (1990). Catabolic plasmids of environmental and ecological significance. *Microbial Ecology, 19*, 1–20.

Schroll, R., Becher, H. H., Dorfler, U., Gayler, S., Grundmann, S., Hartmann, H. P., et al. (2006). Quantifying the effect of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils. *Environmental Science & Technololgy, 40*(10), 3305–3312.

Schroll, R., Brahushi, R., Dorfler, U., Kuhn, S., Fekete, J., & Munch, J. C. (2004). Biomineralisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population. *Environmental Pollution, 127*, 395–401.

Scott, C., Gunjan, P., Carol, J. H., Colin, J. J., Matthew, J. C., Matthew, C. T., et al. (2008). The enzymatic basis for pesticide bioremediation. *Indian Journal of Microbiology, 48*(1), 65–79.

Sethunathan, N., & Yoshida, T. (1973). A *Flavobacterium* sp. that degrades diazinon and parathion. *Canadian Journal of Microbiology, 19*, 873–875.

Shakoori, A. R., Makhdoom, M., & Haq, R. U. (2000). Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. *Applied Microbiology and Biotechnology, 53*, 348–351.

Siddique, T., Okeke, B. C., Arshad, M., & Frankenberger, W. T, Jr. (2003). Biodegradation kinetics of endosulfan by *Fusarium vetricosum* and a *Pandoraea* species. *Journal of Agricultural and Food Chemistry, 51*, 8015–8019.

Skopp, J., Jawson, M. D., & Doran, J. W. (1990). Steady-state aerobic microbial activity as a function of soil water content. *Soil Science Society of America Journal, 54*(6), 1619–1625.

Spyrou, I. M., Karpouzas, D. G., & Menkissoglu-Spiroudi, U. (2009). Do botanical pesticides alter the structure of the soil microbial community. *Microbial Ecology, 58*, 715–727.

Suenaga, H., Mitsuoka, M., Ura, Y., Watanable, T., & Furukawa, K. (2001). Directed evolution of biphenyl dioxygenase: Emergence of enhanced degradation capacity for benzene, toluene and alkyl benzenes. *Journal of Bacteriology, 183*, 5441–5444.

Sukul, P., & Spiteller, M. (2001). Influence of biotic and abiotic factors on dissipating metalaxyl in soil. *Chemosphere, 45*(6), 941–947.

Tejada, M., García, C., Hernández, T., & Gómez, I. (2015). Response of soil microbial activity and biodiversity in soils polluted with different concentrations of Cypermethrin insecticide. *Archives of Environmental Contamination and Toxicology, 69*, 8–19.

Thom, E., Ottow, J. C. G., & Benckiser, G. (1997). Degradation of the fungicide difenoconazole in a silt loam soil as affected by pretreatment and organic amendment. *Environmental Pollution, 96*, 409–414.

Thomas, B., & Parkins, I. B. (1995). *Assimilative capacity of subsurface for the pesticides, atrazine and alachlor and nitrate*. USA: FEDRIP-Data base, National Technical Information Service (NTIS).

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature, 418*(6898), 671–677.

Topp, E. T., & Vallaeys, Soulas G. (1997). Pesticides: Microbial degradation and effects on microorganisms. In J. A. van Elsas (Ed.), *Modem soil microbiology* (pp. 547–573). New York: Marcel Dekker Inc.

UN/DESA. (2008). *Changing unsustainable patterns of consumption and production, Johannesburg plan on implementation of the world summit on sustainable development*. Johannesburg, 2002 (Chapter III).

Van Herwijnen, R., Van de Sande, B. F., Van der Wielen, F. W. M., Springael, D., Govers, H. A. J., & Parsons, J. R. (2003). Influence of phenanthrene and fluoranthene on the degradation of fluorine and glucose by *Sphingomonas* sp. strain LB126 in chemostat cultures. *FEMS Microbiology Ecology, 46*, 105–111.

Verma, J. P., Jaiswal, D. K., & Sagar, R. (2014). Pesticide relevance and their microbial degradation: A state-of-art. *Reviews in Environmental Science & Biotechnology, 13*(4), 429–466.

Verma, P., Verma, P., & Sagar, R. (2013). Variation in N mineralization and herbaceous species diversity due to sites, seasons, and N treatment in a seasonally dry tropical environment of India. *Forest Ecology and Management, 297*, 15–26.

Vollmer, M. D., Hoier, H., Hecht, H. J., Schell, U., Groning, J., Goldman, A., et al. (1998). Substrate specificity of and product formation by muconatecycloisomerases: An analysis of wild type enzyme and engineered variants. *Applied and Environmental Microbiology, 64*, 3290–3299.

Walter-Echols, G., & Lichtenstein, E. P. (1978). Movement and metabolism of ^{14}C-phorate in a flooded soil system. *Journal of Agricultural and Food Chemistry, 26*, 599–604.

Wang, M. C., Gong, M., Zang, H. B., Hua, X. M., Yao, J., Pang, Y. J., et al. (2006). Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. *Journal of Environmental Science and Health Part B, 41*, 399–413.

Wang, M. C., Liu, Y. H., Wang, Q., Gong, M., Hua, X. M., Pang, Y. J., et al. (2008). Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. *Soil Biology & Biochemistry, 40*(3), 778–788.

Wardle, D. A., & Parkinson, D. (1990). Effects of three herbicides on soil microbial biomass and activity. *Plant and Soil, 122*(1), 21–28.

Wood, T. K. (2008). Molecular approaches in bioremediation. *Current Opinion in Biotechnology, 19*, 572–578.

Yan, D. Z., Lui, H., & Zhou, N. Y. (2006). Conversion of *Sphingobium chlorophenolicum* ATCC 39723 to a hexachlorobenzene degrader by metabolic engineering. *Applied and Environmental Microbiology, 72*, 2283–2286.

You, M., & Liu, X. (2004). Biodegradation and bioremediation of pesticide pollution. *Chinese Journal of Ecology, 23*, 73–77.

Yu, Y. L., Chen, Y. X., Luo, Y. M., Pan, X. D., He, Y. F., & Wong, M. H. (2003). Rapid degradation of butachlor in a wheat rhizosphere soil. *Chemosphere, 50*, 771–774.

Zacharia, J. T. (2011). Identity, physical and chemical properties of pesticides. In M. Stoytcheva (Ed.), *Pesticides in the modern world-trends in pesticides analysis* (pp. 1–18). Rijeka: In Tech.

Zhang, W., Xu, J., Dong, F., Liu, X., Zhang, Y., Wu, X., et al. (2014). Effect of tetraconazole application on the soil microbial community. *Environmental Science and Pollution Research, 21*(13), 8323–8332.

An Overview on Microbial Degradation of Lindane

J.M. Saez, A. Alvarez, M.S. Fuentes, M.J. Amoroso and C.S. Benimeli

1 Introduction

The gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), also known as lindane, is a cyclic, saturated and highly chlorinated pesticide as shown in Fig. 1 (Manickam et al. 2008). It is a broad spectrum insecticide, which has been used worldwide for many decades in the last century to control a variety of pests, such as grasshoppers, flies, beetles, mites, termites, ants, and insects affecting cotton and rice crops (Bidlan et al. 2004; Phillips et al. 2005). It has also been applied in human health as scabicide and pediculocide in the form of lotions, creams and shampoos as well as for the treatment of malaria (Bidlan et al. 2004; Lal et al. 2006).

Lindane is produced by photochemical chlorination of benzene with UV light; however, during lindane production, a mixture of several stereoisomers is obtained, (Manickam et al. 2007). It was first applied in the form of technical HCH-a mixture of α-HCH (53–70 %), β-HCH (3–14 %), γ-HCH (10–18 %), δ-HCH (6–10 %), ε-HCH (1–5 %), and also traces of other isomers (Geueke et al. 2013). Later, it was

J.M. Saez (✉) · A. Alvarez · M.S. Fuentes · M.J. Amoroso · C.S. Benimeli
Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET),
Avenida Belgrano y Pasaje Caseros, 4000 Tucumán, Argentina
e-mail: jsaez@proimi.org.ar

A. Alvarez
Facultad de Ciencias Naturales e Instituto Miguel Lillo,
Universidad Nacional de Tucumán, Miguel Lillo 205, 4000 Tucumán, Argentina

M.J. Amoroso · C.S. Benimeli
Universidad del Norte Santo Tomás de Aquino,
9 de Julio 165, 4000 Tucumán, Argentina

M.J. Amoroso
Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán,
Ayacucho 491, 4000 Tucumán, Argentina

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_9

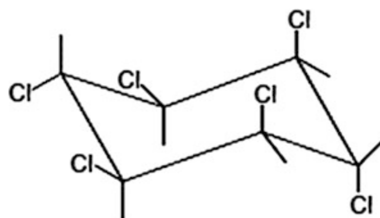


Fig. 1 Chemical structure of lindane

also used in a purified form under the commercial name of lindane (>99 % purity), because the γ isomer is the only one which exhibits insecticidal activity (Nagata et al. 2007). It is estimated that about 600,000 tons of lindane was used between 1950 and 2000, and around 1.7 and 4.8 million tons of HCH residues are still present worldwide (Vijgen 2006).

Some of the most relevant physic-chemical properties of lindane are detailed in Table 1.

It has been shown that lindane and its isomers may cause serious damage to health in the short and long term. In mammals, acute lindane intoxication may cause respiratory dysfunction, generalized trembling, hyper-salivation, and convulsions, which can lead to death also in extreme cases (Pesce et al. 2008). Lindane and other HCH isomers are endocrine disruptors, immune suppressive and they have been also reported as potential carcinogens, teratogenic, genotoxic and mutagenic compounds (ATSDR 2011; Salam and Das 2012). Chronic exposures to these compounds have been linked to renal and hepatic damages, adverse effects on reproductive system, gestation, and nervous system in mammals (Guillén-Jiménez et al. 2012; Salam and Das 2012). Besides, lindane, as all organochlorine pesticides, is known to be persistent in the environment and tends to bioaccumulate along the food chain (Caicedo et al. 2011). Now-a-days, it is considered as Persistent Organic Pollutant according to the Stockholm Convention (Vijgen et al. 2011).

Table 1 Physico-chemical properties of lindane[a]

Lindane	
Chemical formula	$C_6H_6Cl_6$
Molecular weight	290.9
Melting point (°C)	112–113
Boilingpoint (°C)	323.4 at 760 mm Hg
Density (g cm^{-3})	1.89 at 19 °C
Solubility in water (mg L^{-1})	7–10
Solubility in 100 g ethanol (mg)	6.4
Log K_{ow}^{b}	3.72
Log K_{oc}^{c}	3.00–3.57
Vapor pressure (mm Hg)	3.3×10^{-5} at 20–25 °C
EPA toxicity classification	Class II

[a]Adapted from Benimeli et al. (2008); [b]K_{ow}: Octanol-water partition coefficient; [c]K_{oc}: Organic carbon partition coefficient

For these reasons, currently more than 52 countries have banned or severely restricted the use of this xenobiotic compound (Vijgen et al. 2011). However some countries, mainly developing ones, still use it for economic reasons. As the result, new sites are being polluted. Because of its persistence and recalcitrance, lindane and other HCHs residues remain in the environment for a long time and have been recently found in water, soil, sediments, plants, and animals all over the world (Table 2). It was also found in human fluids and tissues, such as blood, amniotic fluid, breast milk and adipose tissue (Luzordo et al. 2009; Herrero-Mercado et al. 2010; Villaamil Lepori et al. 2013).

In this context, scientists, working all over the world, are now involved in developing lindane remediation technologies including physical, chemical, and biological techniques (Salam and Das 2012; Nitoi et al. 2013; Tor et al. 2013; Salam et al. 2013).

Table 2 Some recent reports on the presence of lindane and other HCHs detected in different matrices

Compound	Concentration/range	Sample	Country	Reference
Lindane	1.1–13 ng g^{-1}	Sediment	Portugal	Carvalho et al. (2009)
Lindane	ND–0.75 μg L^{-1}	Water	USA	Flores et al. (2009)
Lindane	0.003 ng mL^{-1}	Human amniotic fluid	Spain	Luzordo et al. (2009)
Lindane	0.45–217 ng g^{-1}	Invertebrates	France	Roche et al. (2009)
HCHs[a]	70.3–149.5 ng g^{-1}	Soil	Argentina	González et al. (2010)
β-HCH	0.002–0.362 mg kg^{-1}	Human adipose tissue	Mexico	Herrero-Mercado et al. (2010)
Lindane	0.02–0.07 μg g^{-1}	Fruits	Ghana	Bempah and Donkor (2011)
HCHs	0.002–59.17 ng g^{-1}	Sediment	Malaysia	Saadati et al. (2012)
Lindane	17.08 ng g^{-1}	Crabs	Nigeria	Alani et al. (2013)
Lindane	0.0001-1.081 μg g^{-1}	Buffalo milk	India	Aslam et al. (2013)
HCHs	23–3220 ng g^{-1}	Birds	South Korea	Hong et al. (2013)
Lindane	1.7 ng g^{-1}	Sediments	Pakistan	Mahmood et al. (2014)
Lindane	1.8–150.5 ng g^{-1}	Fishes	Argentina	Ondarza et al. (2014)
Lindane	ND–256 μg kg^{-1}	Fishes	South Africa	Barnhoorn et al. (2015)
β-HCH	ND–0.049 μg L^{-1}	Groundwater	Italy	Fuscoletti et al. (2015)
Lindane	ND–0.187 mg kg^{-1}	Buffalo milk	Egypt	Shaker and Elsharkawy (2015)

[a]Hexachlorocyclohexane residues with no differentiation of the isomers

The present review compiles the latest updates on microbial transformation of lindane by bacteria, fungi, and algae, its various mechanisms and pathways, factors affecting the degradation process, enzymes involved, and microbial toxicity.

2 Microbial Degradation of Lindane

The key reaction during microbial degradation of halogenated compounds is the removal of the halogen atom, i.e., dehalogenation (Camacho-Pérez et al. 2012). The degree of halogenation of a xenobiotic compound generally determines its toxicity and persistence in the atmosphere, due to the stability of the carbon-halogen bond (Santoyo Telope 2009). Therefore, the dehalogenation of an organochlorine pesticide is the first and the most important step for its degradation (Fetzner and Lingens 1994). In this process the halogen atom is commonly replaced by hydrogen or a hydroxyl group. Halogen removal reduces both recalcitrance to biodegradation and the risk of forming toxic intermediates during subsequent metabolic steps (Camacho-Pérez et al. 2012). Since lindane has six chlorine atoms per molecule, the removal of chlorine ions is a very significant step in its degradation (Nagata et al. 2007).

Different organisms are capable of using lindane or other halogenated compounds as growth substrate, and thus they have a great potential for bioremediation of such compounds. For example, fungi have been described to be capable of degrading various xenobiotics. Among them, white rot species have shown high efficiency in degradation of a wide range of substances. That is why they are of great interest for the development of environment-friendly biotechnological processes (Rigas et al. 2009). Quintero et al. (2007) demonstrated the ability of the white-rot fungus *Bjerkandera adusta* to degrade the four principal isomers of HCH in a slurry system. Besides, *Pleuro tusostreatus*, *Trametes versicolor*, *Hypoxylon fragiforme*, and *Chondrostereum purpureum* are some other white rot-fungi species which have been studied for lindane degradation (Rigas et al. 2009; Ulčnik et al. 2012).

Besides, non-white rot fungi have been also described as HCH-degraders, such as *Fusarium poae* and *F. solani,* isolated from pesticide-contaminated soil, and *Fusarium verticillioides* AT-100, isolated from *Agave tequilana* leaves, which could biodegrade lindane(Sagar and Singh 2011; Guillén-Jiménez et al. 2012).

The use of algae, as remediation agent, may also be attractive and economical because of their low nutrient requirements. Being autotrophic they produce a large biomass, and unlike other microbes, such as bacteria and fungi, they generally do not produce toxic substances (Salam and Das 2012). Zhang et al. (2012) demonstrated that the nitrogen-fixing cyanobacterium *Anabaena azotica* presented tolerance to lindane and removed approximately 49 % of an initial concentration of 0.2 mg $L^{-1}$of lindane in five days. The researchers concluded that the ability of this alga to biodegrade lindane had potential use in bioremediation processes.

Yeasts have been also proved to be efficient for the removal of lindane from aqueous solutions. Salam et al. (2013) isolated a yeast strain *Rhodotorula* sp. VITJzN03 from sorghum cultivation field which was able to completely mineralize 600 mg $L^{-1}$of lindane under aerobic conditions in 10 days. Notably, the authors noted a positive relationship between the release of chloride ions and the increase of yeast biomass as well as degradation of lindane. In addition, other new yeast strain belonging to *Candida* genus was able to use lindane as sole carbon and energy source for its growth in mineral medium, mineralizing 600 mg L^{-1} in less than 6 days, which proves its higher potential on lindane degradation than *Rhodotorula* sp. VITJzN03 (Salam and Das 2014).

Besides, several lindane-degrading bacteria have been also isolated from diverse geographic locations and reported to metabolize or even mineralize lindane under both aerobic and anaerobic conditions. Bacteria, belonging to sphingomonads have been widely studied for lindane degradation. In fact, the degradation pathway of lindane has been completely revealed in *Sphingobium japonicum* (formerly *Sphingomonas paucimobilis*) UT26, and all genes encoding enzymes for the conversion of lindane to metabolites in the tricarboxylic acid cycle have been identified (Nagata et al. 2007). Similarly, *Sphingomonas* sp. NM05, isolated from contaminated soil samples, could utilize around 90 % of an initial concentration of 100 μg $mL^{-1}$of lindane (Manickam et al. 2008). These authors revealed the formation of three intermediate metabolites of lindane degradation pathway. Also, *Sphingobium baderi* sp., isolated from a hexachlorocyclohexane dump located in Czech Republic, was able to degrade α, γ and δ isomers of hexachlorocyclohexane, although no significant degradation activity was observed for the β-isomer (Kaur et al. 2013).

Nevertheless, other Gram-negative genera were also recognized as lindane degrading bacteria, such as *Pandoraea* sp. LIN-3, isolated from an enrichment culture, which could simultaneously degrade α and γ isomers of HCH in both liquid and soil systems (Okeke et al. 2002); and *Xanthomonas* sp., strain isolated from contaminated soil from an industrial site in India, was also capable of biodegrading lindane (Manickam et al. 2007).

Likewise, Gram-positive bacteria have been also reported to remove and degrade lindane. Among these microorganisms, actinobacteria have been studied for their degradative capabilities. *Arthrobacter citreus* BI-100 was able to completely remove 100 mg L^{-1} of lindane in 8 h, but its metabolites were detectable in the medium. After 72 h incubation, complete mineralization was demonstrated, as no γ-HCH metabolite was detected (Datta et al. 2000). De Paolis et al. (2013) also reported the ability of two bacteria *Arthrobacter fluorescens* and *A. giacomelloi*, not directly isolated from a polluted site, which were able to grow in a mineral salt medium containing α-, β-, or γ-HCH (100 mg L^{-1}) as a sole source of carbon. *Microbacterium* ITRC1 has the capacity to degrade all four major isomers of HCH present in both liquid cultures and aged contaminated soil (Manickam et al. 2006). Furthermore, Benimeli et al. (2003, 2006, 2007a, b, 2008) and Fuentes et al. (2010, 2011) isolated and characterized several actinobacteria belonging to *Streptomyces*

and *Micromonospora* genera, which were able to tolerate, remove and/or degrade lindane from culture media, slurries and soils.

Although there are some microorganisms which can completely degrade specific organic pollutants such as lindane; but individual species generally do not contain the complete degradation pathway (Gerhardt et al. 2009). The use of a single culture involves many metabolic limitations, which could be alleviated by using a mixed community of microorganisms (Shong et al. 2012). Microbial consortia can combine catalytic specialties from different species or strains to metabolize pesticide completely. In nature, microorganisms exist in nature as a part of microbial consortia constituted by multiple populations that live and perform complex chemical and physiological processes to allow the survival of the community (Polti et al. 2014).

Several workers have demonstrated that the isolation of consortia, both native and defined from sites previously exposed to a xenobiotic are more likely to succeed in the degradation or removal of such compounds. This can be explained by the fact that prolonged exposure of microorganisms to certain extreme conditions causes selective pressure on the microbes to degrade xenobiotic compound for their survival. Furthermore, exposure of microorganisms to one or more toxic compounds for a long time possibly allowed them to evolve new catabolic enzymes to degrade such compounds (Carrillo-Pérez et al. 2004; Pino et al. 2011).

Murthy and Manonmani (2007) developed a defined microbial consortium consisting of seven *Pseudomonas* spp., and one each of *Burkholderia*, *Flavobacterium* and *Vibrio* genera for the degradation of tech-HCH up to a concentration of 25 ppm. At all concentrations tested, the γ-isomer (lindane) underwent at fastest degradation rate. Also, Fuentes et al. (2011) evaluated lindane removal in 57 mixed cultures of *Streptomyces* spp., and found that degradation was substantially improved when two, three or four strains were used in combination as compared to single cultures.

Other reports also revealed the efficient use of native microbial consortia to degrade lindane and other HCH isomers. Elcey and Kunhi (2010) isolated a lindane degrading consortium from a sugar cane field having a long history of technical-HCH application. The consortium could mineralize 300 $\mu g\ mL^{-1}$ of lindane after 108 h of acclimation in presence of the substrate, since no apparent accumulation of intermediary metabolites was observed.

In addition to microbes, plants have been also reported to possess remarkable ability to remove or immobilize various isomers of HCH. Becerra-Castro et al. (2013) evaluated HCH removal by *Cytisus striatus*, a tolerant leguminous shrub, in association with different microbial inoculant treatments and confirmed that HCH dissipation was enhanced in planted pots. In another study, Alvarez et al. (2012) detected specific dechlorinase activity in root exudates from *Zea mays* which, in association with *Streptomyces* strains, improved lindane removal from the liquid medium.

3 Factors Affecting Lindane Degradation Process

A number of physical, chemical, and biological factors have been reported to affect lindane biodegradation, i.e. initial lindane concentration and bioavailability, presence of oxygen, temperature, pH, biomass concentration, and composition of the medium, etc. (Salam and Das 2012).

3.1 Additional Carbon Sources

Several researchers have demonstrated that carbon sources, other than the target pollutant, may influence on its degradation, but this is also governed by other factors like microbial community, concentration of pollutants and nutrients etc. Therefore, an additional carbon source does not always stimulate the degradation process, but in some cases, it may be indifferent, and in others it may limit the degradation process (Pino et al. 2011).

Co-metabolism is a very important process for the removal of certain xenobiotics (García-Rivero and Peralta-Pérez 2008). For instance, Benimeli et al. (2007a) demonstrated that glucose and lindane were simultaneously consumed by *Streptomyces* sp. M7, and the presence of high concentrations of glucose stimulated lindane removal and biomass yield. Guillén-Jiménez et al. (2012) suggested that the addition of agave leaves to the culture medium improved the fungal lindane degradation by biostimulation. Similarly, Alvarez et al. (2012) evaluated lindane removal by *Streptomyces* strains in the presence of root exudates as additional carbon source. Under this condition, *Streptomyces* sp. A5 and M7 caused 55 and 35 % of lindane removal, respectively. Hence, co-metabolism may be an important plant/microbe interaction to induce bioremediation. In addition, root exudates may enhance pesticide biodegradation by increasing its bioavailability, as reported by Calvelo Pereira et al. (2006). These authors showed an increase in the aqueous solubility of different HCH isomers (including lindane) and a reduction in their concentration in the rhizosphere of *Avena sativa* and *Cytisus striatus* as compared to bulk soil.

3.2 Other Nutritional Factors

Guillén-Jiménez et al. (2012) evaluated the effect of some nutritional factors on lindane biodegradation by *Fusarium verticillioides* AT-100. They found that lindane degradation was favoured in the presence of limited amounts of nitrogen and phosphorus, and without surfactant in the medium. The use of higher concentrations of lindane, copper, and yeast extract also enhanced the biodegradation process. Nagpal et al. (2008) also demonstrated that lindane degradation by

Conidiobolus 03-1-56 was enhanced under limited levels of nitrogen. Although nitrogen is an essential element for the growth of microorganisms; at low concentrations, it may induce the expression of laccases and other enzymes, which degrade lignin and other xenobiotic pollutants (Guillén-Jiménez et al. 2012).

3.3 Inoculum Size

The inoculum size can also influence the biodegradation efficiency of highly toxic compounds. Some reports have demonstrated that the use of a larger inoculum may enhance the lindane biodegradation efficiency (Guillén-Jiménez et al. 2012). However, lindane removal and/or transformation are not always proportional to the inoculum size. Saez et al. (2014) observed higher lindane removal from soil slurry employing an immobilized *Streptomyces* consortium at 10^7 CFU g^{-1} of inoculum than smaller inoculum size. However, employing higher cell densities had no additional benefits on the pesticide removal. Similar findings were reported by Fuentes et al. (2010), who demonstrated that the inoculum and lindane removal are not in direct proportion. Lindane removal by *Streptomyces* sp. M7 in soil samples increased, when the inoculum concentration augmented from 0.5 to 2 g cells kg^{-1} soil. However, lindane removal decreased at 4 g cells kg^{-1} soil. Salam and Das (2014) also noted that increasing the inoculum size (from 0.02 to 0.06 mg L^{-1}) of *Candida* sp. VITJzN04 strain promoted the degradation process from 40 to 100 % at the same incubation time. However, when the initial inoculum was increased further, there was no significant impact on the degradation rate.

3.4 Lindane Concentration

The concentration of the target pollutant is an important factor which affects the biomass production as well as the degradation rates. Low concentration of pollutant might not be able to induce the degradative enzymes activity, while too high levels may be toxic to the microorganisms (Awasthi et al. 2000).

For instance, *Rhodotorula* VITJzN03 was able to mineralize 100 % of 600 mg L^{-1} of lindane, but concentrations beyond it inhibited the growth of the yeast in the liquid medium (Salam et al. 2013). Pesce and Wunderlin (2004) showed that a bacterial consortium increased the lindane degradation rate when exposed to higher lindane concentrations, but when its level increased from 0.069 to 0.412 mM, the biodegradation efficiency decreased from 100 to 83.3 %. In another study, Guillén-Jiménez et al. (2012) observed that increasing lindane concentration might facilitate its removal. Okeke et al. (2002) studied the ability of a *Pandoreae* sp. strain to remove lindane in liquid and soil slurry systems. Their results indicated that the rates and extent of lindane removal increased with increasing concentrations up to 150 mg L^{-1}, and then declined at 200 mg L^{-1}.

There is evidence that pesticide biodegradation rates in soil follow first-order kinetics and are concentration dependent. As a consequence, lindane removal rates might decrease, as the concentrations are reduced. HCH may also affect soil microbial populations, stimulate growth of certain microorganisms and exert toxic effects, inhibiting growth of others (Phillips et al. 2005). In this sense, Saez et al. (2014) reported that the maximum removal was obtained in soil slurries spiked with the highest lindane concentration (50 mg kg^{-1}).

3.5 pH

It is one of the most important factors affecting lindane degradation, as the reduction in pH may hinder the growth of the degraders during the process of degradation (Salam and Das 2012). The effect of pH on lindane degradation has been studied in *Pandoraea* sp. The optimum pH for microbial growth and biodegradation of lindane in soil slurries was 9.0 (Okeke et al. 2002). Elcey and Kunhi (2010) reported that an acclimated consortium could degrade γ-HCH at a wide range of pH of 3–9, while the optimum was at more neutral pH range, between pH 6 and 8. In contrast, Murthy and Manonmani (2007) reported that at pH 4, there was practically no degradation of lindane by a defined consortium consisting of 10 microorganisms. Thus, pH of the medium had a substantial effect on the survival of the members of the consortium. With an increase in pH towards neutrality, the microbial survivability improves gradually. Benimeli et al. (2007b) also evaluated the effect of initial pH on lindane removal by *Streptomyces* sp. M7 in soil extract, and determined that the highest pesticide removal (70 %) was recorded at an initial pH of 7. In more recent studies, Salam et al. (2013) reported that the optimum pH for lindane degradation by *Rhodotorula* sp. VITJzN03 was found to be pH 6.

3.6 Temperature

In general, the optimum temperature for biodegradation of HCH isomers ranges from 25 to 30 °C in soil, soil slurry, and bacterial cultures. An increase in temperature (within limits) might enhance HCH removal simply by increasing biological activity, or might also affect biodegradation by enhancing bioavailability through reduced sorption (Phillips et al. 2005).

Elcey and Kunhi (2010) evaluated lindane degradation at a wide range of temperatures, and demonstrated that chloride ions release occurred even at 5 and 60 °C (8 and 18 %, respectively), whereas optimum chloride release was at 30–35 °C. Similar results were observed by Benimeli et al. (2007b) who obtained maximum lindane removal by *Streptomyces* sp. M7 at 30 °C. Also, rapid degradation of lindane was reported by *Sphingobium* strains at low temperature (4 °C) (Zheng et al. 2011).

3.7 Bioavailability

Several factors affect volatility and bioavailability of lindane (vapor pressure, soil organic matter, and moisture) and hence, can influence its biodegradation (Salam and Das 2012). Sorption of lindane to the surface of soil particles reduces mobility, but may increase the proximity of contaminants to surface-bound microorganisms. Thus, the factors, that affect solubility and sorption of lindane, influence their movement within the soil matrix, thereby affecting their bioavailability and biodegradation. Lindane can also be volatilized through air pockets of the soil or escape from the surface affecting its concentrations in the solid and liquid phases of the soil and also its bioavailability (Phillips et al. 2005).

Soil properties and composition can also affect lindane degradation. The organic carbon fraction of soil tends to decrease the bioavailability of organic compounds (Becerra-Castro et al. 2013). In this connection, Vlčková and Hofman (2012) demonstrated that soil properties crucially affect the bioavailability of persistent organic pollutants, such as lindane, DDT, phenanthrene, and pyrene. They found that an increase in the total organic carbon content of the soil caused a decrease in the bioavailability of contaminants. Besides, it has been suggested that a higher silt content in soil results in higher moisture retention, which may enhance bioavailability by inhibiting option of γ-HCH (Phillips et al. 2005). Roy et al. (2000) also observed that with increasing water content of soil, organic material becomes hydrophilic, so the adsorption of hydrophobic compounds decreases due to hydration of the adsorbent surfaces and thus lowers the accessibility of the adsorption sites.

4 Mechanism and Pathways of Lindane Degradation

For a long time it was assumed that lindane degradation pathway was largely an anaerobic process, but it was observed that it could be carried out in both aerobic and anaerobic conditions (Phillips et al. 2005; Lal et al. 2010). The existing reports revealed that different biodegradation routes occurred for lindane under aerobic and anaerobic conditions.

4.1 Anaerobic Degradation of Lindane

Anaerobic biodegradation of lindane is known for about 50 years. Quintero et al. (2005) proposed a route for the degradation of lindane and α-HCH isomer under anaerobic conditions based on their results obtained in liquid and soil slurry cultures (Fig. 2). This hypothetical anaerobic biodegradation pathway is initiated with

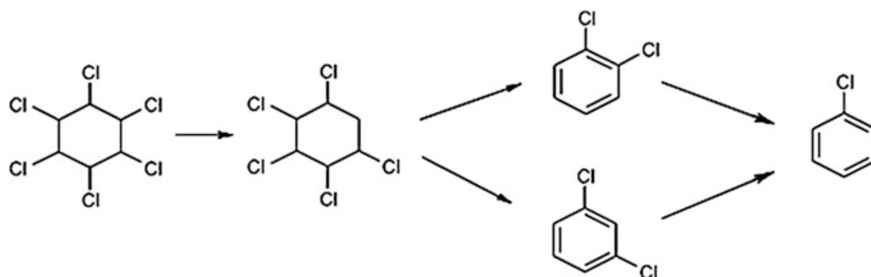


Fig. 2 Degradation pathway for lindane under anaerobic conditions (Quintero et al. 2005)

dechlorination of the HCH isomers to form pentachlorocyclohexane, and subsequently 1,2- and 1,3-dichlorobenzene (DCB), and finally chlorobenzene (CB).

In subsequent studies, Quintero et al. (2006) reported the anaerobic degradation of lindane and other three isomers of HCH in an anaerobic reactor containing soil slurry cultures. They observed that during the different degradation phases, traces of diverse intermediate and end-products were detected, such as pentachlorocyclohexane, tetrachlorocyclohexene (TCCH),1,2,3-trichlorobenzene (1,2,3-TCB), 1,3-DCB, and CB. Low concentrations of these compounds were detected and the prolonged operational period of the reactor indicates that intermediate compounds were not accumulated and they were further degraded to CB as end-product in this degradation process.

Other authors also reported anaerobic degradation of lindane, with detection of intermediate metabolites. For instance, Badea et al. (2009) demonstrated that lindane was dechlorinated by a mixed culture of *Desulfococcus multivorans* DSM 2059 and *Desulfovibrio gigas* DSM 1382in four weeks, and metabolites, such as γ-3,4,5,6-TCCH, CB, and benzene, were formed.

Elango et al. (2011) demonstrated that γ-HCH was used as a terminal electron acceptor by an anaerobic enrichment culture and got reduced to a mixture of benzene and CB. The addition of vancomycin into culture significantly slowed the rate of γ-HCH dechlorination, suggesting that Gram-positive organisms were responsible for the anaerobic dechlorination of lindane.

4.2 Aerobic Degradation of Lindane

The aerobic degradation pathway of γ-HCH was completely revealed in bacterial strain *Sphingobium japonicum* UT26 (formerly *Sphingomonas paucimobilis* UT26) (Nagata et al. 2007). In this route, lindane is transformed to 1,2,4-TCB; 2,5-dichlorophenol (2,5-DCP); and 2,5-dichlorohydroquinone(2,5-DCHQ), via different reactions which is referred as upstream pathway. Among these three metabolites, only 2,5-DCHQ is further metabolized by *S. japonicum* UT26, through a series of steps (referred as the downstream pathway) to succinyl-CoA and

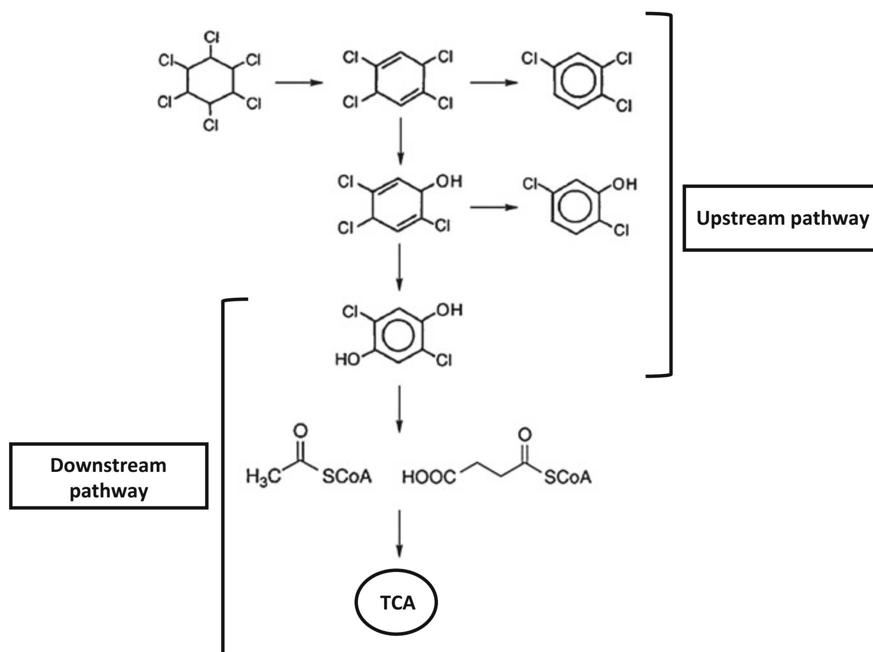


Fig. 3 Lindane degradation pathway in *Sphingobium japonicum* UT26 (adapted from Nagata et al. 2007)

acetyl-CoA, which are further metabolized in the citrate/tricarboxylic acidcycle (TCA) (Fig. 3). In a recent study, the formation of γ-pentachlorocyclohexene (γ-PCCH), 2,5-DCP, and 2,5-dichloro-2,5-cyclohexadiene-1,4-diol(2,5-DDOL)was also reported when other bacterial strains, such as *S. indicum* B90A and *S. lucknowense* F2, were grown in the presence of lindane (Geueke et al. 2013).

Besides, aerobic degradation of lindane was also studied in other genera, which suggested the same and also different intermediate metabolites from those reported by Nagata et al. (2007). For instance, two metabolic intermediates of lindane, i.e., γ-PCCH and 2,5-dichlorobenzoquinone (2,5-DCBQ), were identified during γ-HCH biodegradation by *Xanthomonas* sp. (Manickam et al. 2007). While the γ-PCCH had been previously reported for *S. japonicum* UT26, the 2,5-DCBQ was observed as a novel metabolite in lindane degradation pathway.

Cuozzo et al. (2009) also detected γ-PCCH and 1,3,4,6-tetrachloro-cyclohexadiene(1,3,4,6-TCDN)in cell-free extract of *Streptomyces* sp. M7 grown with lindane, such as the first and second products of the lindane catabolism in the catabolic pathway proposed by Nagata et al.(2007). This could suggest that the aerobic degradation route of lindane might not be very different in Gram-positive and Gram-negative bacteria. However, on the other hand, Datta et al. (2000) identified six metabolic intermediates of γ-HCH produced by *Arthrobacter citreus* BI-100 at different periods of growth, i.e. γ-PCCH, TCCH, trichlorocyclohexadiene

(TCCD), 2-chlorophenol (2-CP), phenol, and catechol. It is noteworthy that the aerobic degradation of lindane by *A. citreus* BI-100 does not produce the 1,3,4,6-TCDN as transient dehydrochlorination product of γ-PCCH; while it does occur during the metabolism of γ-HCH in *S. japonicum* UT26 and *Streptomyces* sp. M7 (Nagata et al. 2007; Cuozzo et al. 2009). It is also worth mentioning that the formation of TCCD by *A. citreus* BI-100 occurred in contrast to the production of 1,2,4-TCB in *S. japonicum* UT26 (Nagata et al. 2007). Moreover, lindane degradation pathway in *S. japonicum* UT26 does not produce 2-CP, catechol, nor phenol, in contrast to the degradation pathway of γ-HCH by *A. citreus* BI-100. Hence, it can be concluded that some metabolites produced by this microorganism during lindane degradation pathway are quite different from those produced by other microorganisms (Datta et al. 2000).

In addition, De Paolis et al. (2013) detected the formation of PCCH and TCCH in cultures of *Arthrobacter fluorescens* and *A. giacomelloi* grown with γ-HCH (100 mg L^{-1}) as sole source of carbon. In comparison with metabolites produced by *A. citreus* BI-100 (Datta et al. 2000), neither phenol or chlorophenol, nor catechol were detected after 24 h of incubation. This finding suggests that *A. fluorescens* and some other *Arthrobacter* species might be able to utilize and degrade phenolic compounds or its enzymatic system does not produce phenols. Thus, they presented a different degradation pathway for lindane from that described by Datta et al. (2000).

In a recent study, Salam and Das (2014), based on six metabolites identified during lindane degradation by *Candida* VITJzN04, proposed a possible degradation pathway in which the parent compound was successively dechlorinated to form γ-PCCH and trichloro-2,5-cyclohexadiene-1-ol (TCCH-1-ol). Further, via multi-step reduction and dechlorination, 2,5-DCHQ is formed and then reduced to 2-chlorohydroquinone (2-CHQ). The closed ring is then opened and hydrolysed to produce hydroxymuconicsemialdehyde (HMSA), which is oxidized to form maleyl acetate to be incorporated directly into TCA. While γ-PCCH and benzoic acid derivatives are some of the major metabolites reported during lindane degradation by fungi (Guillén-Jiménez et al. 2012), the presence of metabolites such as, derivates of hydroquinone and HMSA implies that different enzymatic mechanisms exist for lindane degradation.

5 Genes and Enzymes Involved in Lindane Degradation Process

Some genes encoding for lindane degradation enzymes have already been cloned, sequenced and characterized. These genes, called *lin* genes, are highly conserved in diverse genera of bacteria, including Gram-positive groups, occurring in various environmental conditions (Girish and Kunhi 2013).

The *lin* genes were initially identified and characterized in *Sphingobium japonicum* UT26 (Nagata et al. 1999). In lindane degradation pathway proposed for *S. japonicum* UT26, three enzymes are involved in the upstream pathway: a dehydrochlorinase (LinA), a halidohydrolase (LinB), and a dehydrogenase (LinC). Subsequentely, in downstream pathway, other enzymatic activities are involved, such as a reductive dechlorinase (LinD), a ring-cleavage dioxygenase (LinE), a maleylacetate reductase (LinF), a 3-oxoadipate CoA transferase (LinGH), and a β-ketoadipyl CoA thiolase (LinJ), as shown in Fig. 4.

The *linA*, *linB*, and *linC* genes, encoding for LinA, LinB, and LinC, respectively, are constitutively expressed (Nagata et al.1999); while *linD* and *linE* genes form an operon, and their transcription is induced by a regulator named LinR in the presence of hydroquinone-type compounds, such as chlorohydroquinone, hydroquinone, and 2,5-dichlorohydroquinone (Miyauchi et al. 2002).

Dehalogenases are key enzymes for the degradation of various halogenated compounds. Since lindane has six chlorine atoms per molecule, different dehalogenases are involved in its degradation process. Among these dehalogenases, LinA and LinB have been the focus of enzymatic analysis and extensively characterized (Nagata et al. 2007).

LinA and LinB catalyze two successive reactions separately, and in *S. japonicum* UT26, they are both located in the periplasm (Nagata et al. 1999), although a homologue of LinA in *Rhodanobacter lindaniclasticus* appears to be secreted extracellularly (Nalin et al. 1999).

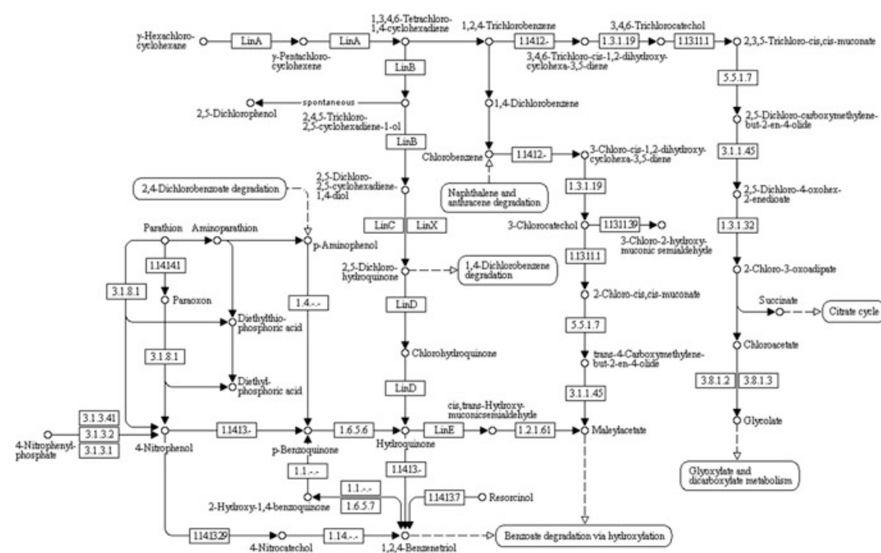


Fig. 4 Enzymes involved in lindane degradation pathway and intermediate metabolites (KEGG 2014)

In *S. japonicum* UT26, the 465-bp *linA* gene encodes a 155 amino acid protein with a predicted MW of 17.2 kDa (Nagata et al. 1993). The *S. indicum* B90A has two different variants of LinA, LinA1 and LinA2. One of them, LinA2, is identical to LinA-UT26, whereas LinA1 is 88 % identical to LinA2-B90A/LinA-UT26 (Kumari et al. 2002).

The 885-bp *linB* gene of *S. japonicum* UT26 encodes a haloalkane dehalogenase, which belongs to the α/β-hydrolase family. This 32 kDa polypeptide has 29.3 % amino acid sequence identity and similar properties (conserved residues in the putative active site) to the haloalkane dehalogenase, DhlA, of *Xanthobacter autotrophicus* GJ10 (Nagata et al. 1993, 2007).

LinA has narrow substrate specificity, although there is experimental evidence that it can also dehydrochlorinate other HCH isomers besides lindane, such as α and δ (Wu et al. 2007; Lal et al. 2010). In contrast, LinB presents broad substrate specificity and is demonstrated to hydrolytically dechlorinate only β- and δ-HCH in all strains examined (Nagata et al. 2005; Geueke et al. 2013).

Manickam et al. (2006) amplified and sequenced two genes involved in the degradation pathway of γ-HCH in *Microbacterium* sp. ITRC1, homologous to the corresponding *linB* and *linC* of *S. japonicum* UT26. The *linB* 800-bp PCR product was identical to the reported hydrolytic dehalogenase of *S. japonicum* UT26. In *linC* amplification product, five of 423 nucleotides were different when compared to UT26, which resulted in changes in two of a total of 141 amino acid residues. In the *linC* gene of *Microbacterium* sp., a phenylalanine was substituted by a serine, while a glutamate was replaced by a glycine.

On the other hand, several studies reported the presence of dehalogenase activity and/or chloride ions release in some strains, specially belonging to *Streptomyces* genus (Benimeli et al. 2006; Cuozzo et al. 2009; Fuentes et al. 2010, 2011). Furthermore, in a recent study, Salam and Das (2014) reported for the first time the involvement of enzymes viz. lindane dechlorinase and lindane dehalogenase during lindane degradation by yeast species.

6 Microbial Toxicity of Lindane and Its Degradation Products

It has been reported that xenobiotics and/or their metabolites may be toxic to bacteria. Furthermore, the oxidative conversion of several halogenated organic compounds may lead to the production of acylhalides or 2-haloaldehydes. The latter are very reactive products due to their electrophilicity and may cause cellular damage (Camacho-Pérez et al. 2012).

Endo et al. (2006) reported that the growth of *Sphingobium japonicum* UT26 in rich medium was inhibited by γ-HCH. However, this growth inhibition was not observed in a mutant that lacked the initial or second enzymatic activity for γ-HCH degradation. Consequently, they demonstrated that two metabolites of γ-HCH,

2,5-DCP and 2,5-DCHQ, showed a direct toxic effect on UT26 and others sphingomonads strains. Although both compounds are toxic to UT26, 2,5-DCP is thought to be the mainly responsible for the growth inhibition, since 2,5-DCHQ disappears by LinD activity, but 2,5-DCP accumulates during γ-HCH degradation. Later, Endo et al. (2007) identified the genes encoding a putative ABC-type transporter essential for the γ-HCH utilization in UT26, involved in the γ-HCH utilization by conferring tolerance to toxic metabolite(s) of the cells, especially to 2,5-DCP.

On the other hand, Quintero et al. (2006) evaluated the potential toxicity of the HCH isomers on methanogenic bacteria in batch assays, and observed variations while using different xenobiotic concentrations. The addition of 250 mg HCH kg^{-1} soil had a variable negative effect on the methanogenic activity dependent on the isomer, being the γ-isomer the one which presented the highest toxicity (95 % methanogenic activity reduction respect to control). A higher concentration of 500 mg of HCH kg^{-1} soil resulted in total inhibition of the methanogenic activity except for β-HCH.

El-Bestawy et al. (2007) reported different lindane effects on the growth of cyanobacterial species depending on the concentration and the exposure time. The higher concentration (10 ppm) induced slightly higher toxicity than to the lower concentration (5 ppm), regardless of the species or the exposure time. Also, a significant correlation between lindane toxicity and exposure time was detected for all the tested species.

7 Future Prospectives

The biodegradation pathway of lindane was completely elucidated in *Sphingobium japonicum* UT26 and also extensively studied in other members of sphingomonads. Although, some metabolic intermediates have been identified in aerobic systems with Gram-positive bacteria; but there is scarce information regarding the metabolic pathway for lindane degradation and the genes and enzymes involved in this process for Gram-positive bacteria, specifically actinobacteria.

Furthermore, several factors affecting lindane degradation process have been studied, such as pH, temperature, soil properties, lindane concentration, and bioavailability, etc. Nevertheless, the degradation potential observed under laboratory conditions should be compared to in situ conditions to assess the success of a bioremediation. In fact, there is still a lack of information available on bioremediation technology applicable for actual field-scale treatment of soil and liquid systems contaminated with lindane and other HCH isomers. Also, more research is needed to understand the interactions of lindane-degrading microorganisms with the environment and the autochthonous microflora.

8 Conclusions

This article provides updated information on lindane biodegradation by different microorganisms such as bacteria, fungi, and algae, under both aerobic and anaerobic conditions. The investigation demonstrates that although only 65 years have been since the first release of lindane into the environment, certain microorganisms have acquired the ability to transform and degrade this compound. In this sense, lindane-degrading microorganisms may be used both as models to understand how they have evolved in order to adapt to the environment and also as a biotechnological tool to bioremediate polluted environments.

Acknowledgments This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Secretaria de Ciencia, Arte e Innovación Tecnológica (SCAIT), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

References

Alani, R., Drouillard, K., Olayinka, K., & Alo, B. (2013). Bioaccumulation of organochlorine pesticide residues in fish and invertebrates of Lagos Lagoon, Nigeria. *American Journal of Scientific and Industrial Research, 4*, 22–30.

Alvarez, A., Yanez, M. L., Benimeli, C. S., & Amoroso, M. J. (2012). Maize plants (*Zea mays*) root exudates enhance lindane removal by native *Streptomyces* strains. *International Biodeterioration and Biodegradation, 66*, 14–18.

Aslam, M., Rais, S., & Alam, M. (2013). Quantification of organochlorine pesticide residues in the buffalo milk samples of Delhi City, India. *Journal of Environmental Protection, 4*, 964–974.

ATSDR (2011) Agency for Toxic Substances and Disease Registry. Hexachlorocyclohexane (HCH). Atlanta, USA.

Awasthi, N., Ahuja, R., & Kumar, A. (2000). Factors influencing the degradation of soil applied endosulfan isomers. *Soil Biology and Biochemistry, 32*, 1697–1705.

Badea, S. L., Vogt, C., Weber, S., Danet, A. F., & Richnow, H. H. (2009). Stable isotope fractionation of gamma-hexachlorocyclohexane (lindane) during reductive dechlorination by two strains of sulfate-reducing bacteria. *Environmental Science and Technology, 43*, 3155–3161.

Barnhoorn, I. E. J., van Dyk, J. C., Genthe, B., Harding, W. R., Wagenaar, G. M., & Bornman, M. S. (2015). Organochlorine pesticide levels in *Clarias gariepinus* from polluted freshwater impoundments in South Africa and associated human health risks. *Chemosphere, 120*, 391–397.

Becerra-Castro, C., Kidd, P. S., Rodríguez-Garrido, B., Monterroso, C., Santos-Ucha, P., & Prieto-Fernández, A. (2013). Phytoremediation of hexachlorocyclohexane (HCH) contaminated soils using *Cytisus striatus* and bacterial inoculants in soils with distinct organic matter content. *Environmental Pollution, 178*, 202–210.

Bempah, C. K., & Donkor, A. K. (2011). Pesticide residues in fruits at the market level in Accra Metropolis, Ghana, a preliminary study. *Environmental Monitoring and Assessment, 175*, 551–561.

Benimeli, C. S., Amoroso, M. J., Chaile, A. P., & Castro, G. R. (2003). Screening of organochlorine pesticides removal by aquatic streptomycetes. *Bioresource Technology, 89*, 133–138.

Benimeli, C. S., Castro, G. R., Chaile, A. P., & Amoroso, M. J. (2006). Lindane removal induction by *Streptomyces* sp. M7. *Journal of Basic Microbiology, 46*, 348–357.

Benimeli, C. S., Castro, G. R., Chaile, A. P., & Amoroso, M. J. (2007a). Lindane uptake and degradation by aquatic *Streptomyces* sp. strain M7. *International Biodeterioration and Biodegradation, 59*, 148–155.

Benimeli, C. S., Fuentes, M. S., Abate, C. M., & Amoroso, M. J. (2008). Bioremediation of lindane-contaminated soil by *Streptomyces* sp. M7 and its effects on *Zea mays* growth. *International Biodeterioration and Biodegradation, 61*, 233–239.

Benimeli, C. S., Gonzalez, A. J., Chaile, A. P., & Amoroso, M. J. (2007b). Temperature and pH effect on lindane removal by *Streptomyces* sp. M7 in soil extract. *Journal of Basic Microbiology, 47*, 468–473.

Bidlan, R., Afsar, M., & Manonmani, H. K. (2004). Bioremediation of HCH-contaminated soil: Elimination of inhibitory effects of the insecticide on radish and green gram seed germination. *Chemosphere, 56*, 803–811.

Caicedo, P., Schroder, A., Ulrich, N., Schroter, U., Paschke, A., Schuurmann, G., et al. (2011). Determination of lindane leachability in soil–biosolid systems and its bioavailability in wheat plants. *Chemosphere, 84*, 397–402.

Calvelo Pereira, R., Camps-Arbestain, M., Rodríguez Garrido, B., Macías, F., & Monterroso, C. (2006). Behaviour of α-, β-, γ-, and δ-hexachlorocyclohexane in the soil-plant system of a contaminated site. *Environmental Pollution, 144*, 210–217.

Camacho-Pérez, B., Rios-Leal, E., Rinderknecht-Seijas, N., & Poggi-Varaldo, H. M. (2012). Enzymes involved in the biodegradation of hexachlorocyclohexane: A mini review. *Journal of Environmental Management, 95*, S306–S318.

Carrillo-Pérez, E., Ruiz-Manríquez, A., & Yeomans-Reina, H. (2004). Isolation, identification and evaluation of a mixed culture of microorganisms capable of degrading DDT (in Spanish). *Revista Internacional de Contaminación Ambiental, 20*, 69–75.

Carvalho, P. N., Rodrigues, P. N. R., Basto, M. C. P., & Vasconcelos, M. T. S. D. (2009). Organochlorine pesticides levels in Portuguese coastal areas. *Chemosphere, 75*, 595–600.

Cuozzo, S. A., Rollan, G. G., Abate, C. M., & Amoroso, M. J. (2009). Specific dechlorinase activity in lindane degradation by *Streptomyces* sp. M7. *World Journal of Microbiology and Biotechnology, 25*, 1539–1546.

Datta, J., Maiti, A. K., Modak, D. P., Chakrabartty, P. K., Bhattacharyya, P., & Ray, P. K. (2000). Metabolism of γ-hexachlorocyclohexane by *Arthrobacter citreus* strain BI-100: Identification of metabolites. *Journal of General and Applied Microbiology, 46*, 59–67.

De Paolis, M. R., Lippi, D., Guerriero, E., Polcaro, C. M., & Donati, E. (2013). Biodegradation of α-, β-, and γ-Hexachlorocyclohexane by *Arthrobacter fluorescens* and *Arthrobacter giacomelloi*. *Applied Biochemistry and Biotechnology, 170*, 514–524.

Elango, V., Kurtz, H. D, Jr., Anderson, C., & Freedman, D. L. (2011). Use of γ-hexachlorocyclohexane as a terminal electron acceptor by an anaerobic enrichment culture. *Journal of Hazardous Materials, 197*, 204–210.

El-Bestawy, E. A., Abd El-Salam, A. Z., & Abd El-Rahman, H. M. (2007). Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents. *International Biodeterioration and Biodegradation, 59*, 180–192.

Elcey, C. D., & Kunhi, A. A. M. (2010). Substantially enhanced degradation of hexachlorocyclohexane isomers by a microbial consortium on acclimation. *Journal of Agriculture and Food Chemistry, 58*, 1046–1054.

Endo, R., Ohtsubo, Y., Tsuda, M., & Nagata, Y. (2006). Growth inhibition by metabolites of γ-hexachlorocyclohexane in *Sphingobium japonicum* UT26. *Bioscience, Biotechnology, and Biochemistry, 70*, 1029–1032.

Endo, R., Ohtsubo, Y., Tsuda, M., & Nagata, Y. (2007). Identification and characterization of genes encoding a putative ABC-type transporter essential for the utilization of γ-hexachlorocyclohexane in *Sphingobium japonicum* UT26. *Journal of Bacteriology, 189*, 3712–3720.

Fetzner, S., & Lingens, F. (1994). Bacterial dehalogenases: Biochemistry, genetics and biotechnological applications. *Microbiological Reviews, 58*, 641–685.

Flores, B., Camarena, C., Ren, J., Krishnamurthy, S., & Belzer, W. (2009). Assessment of organochlorine pesticide levels in Manadas Creek, an urban tributary of the Rio Grande in Laredo, Texas. *Archives of Environmental Contamination and Toxicology, 57*, 11–20.

Fuentes, M. S., Benimeli, C. S., Cuozzo, S. A., Saez, J. M., & Amoroso, M. J. (2010). Microorganisms capable to degrade organochlorine pesticides. In A. Méndez-Vilas (Ed.), *Current research, technology and education topics in applied microbiology and microbial biotechnology* (pp. 1255–1264). Badajoz: Formatex.

Fuentes, M. S., Saez, J. M., Benimeli, C. S., & Amoroso, M. J. (2011). Lindane biodegradation by defined consortia of indigenous *Streptomyces* strains. *Water, Air, and Soil Pollution, 222*, 217–231.

Fuscoletti, V., Achene, L., Gismondi, F., Lamarra, D., Lucentini, L., Spina, S., et al. (2015). Presence of epsilon HCH together with four other HCH isomers in drinking water, groundwater and soil in a former lindane production site. *Bulletin of Environment Contamination and Toxicology, 95*, 108–115.

García-Rivero, M., & Peralta-Pérez, M. R. (2008). Cometabolism in the biodegradation of hydrocarbons. *Revista Mexicana de Ingeniería Biomédica, 7*, 1–12.

Gerhardt, K. E., Huang, X. D., Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Science, 176*, 20–30.

Geueke, B., Garg, N., Ghosh, S., Fleischmann, T., Holliger, C., Lal, R., et al. (2013). Metabolomics of hexachlorocyclohexane (HCH) transformation: Ratio of LinA to LinB determines metabolic fate of HCH isomers. *Environmental Microbiology, 15*, 1040–1049.

Girish, K., & Kunhi, A. A. M. (2013). Microbial degradation of gamma-hexachlorocyclohexane (lindane). *African Journal of Microbiology Research, 7*, 1635–1643.

González, M., Miglioranza, K. S. B., Aizpún, J. E., Isla, F. I., & Pena, A. (2010). Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia). *Chemosphere, 81*, 351–358.

Guillén-Jiménez, F. M., Cristiani-Urbina, E., Cancino-Díaz, J. C., Flores-Moreno, J. L., & Barragán-Huerta, B. E. (2012). Lindane biodegradation by the *Fusarium verticillioides* AT-100 strain, isolated from *Agave tequilana* leaves: Kinetic study and identification of metabolites. *International Biodeterioration and Biodegradation, 74*, 36–47.

Herrero-Mercado, M., Waliszewski, S. M., Valencia-Quintana, R., Caba, M., Hernández-Chalate, F., García-Aguilar, E., et al. (2010). Organochlorine pesticide levels in adipose tissue of pregnant women in Veracruz, Mexico. *Bulletin of Environmental Contamination and Toxicology, 84*, 652–656.

Hong, S. H., Shim, W. J., Han, G. M., Ha, S. Y., Jang, M., Rani, M., et al. (2013). Levels and profiles of persistent organic pollutants in resident and migratory birds from an urbanized coastal region of South Korea. *Science of the Total Environment, 1*, 1463–1470.

Kaur, J., Moskalikova, H., Niharika, N., Sedlackova, M., Hampl, A., Damborsky, J., et al. (2013). *Sphingobium baderi* sp. nov., isolated from a hexachlorocyclohexane dump site. *International Journal of Systematic and Evolutionary Microbiology, 63*, 673–678.

KEGG. (2014). Kyoto encyclopedia of genes and genomes. KEGG Pathway Database.

Kumari, R., Subudhi, S., Suar, M., Dhingra, G., Raina, V., Dogra, C., et al. (2002). Cloningand characterization of *lin* genes responsible for the degradationof hexachlorocyclohexane isomers by *Sphingomonaspaucimobilis* strain B90. *Applied and Environment Microbiology, 68*, 6021–6028.

Lal, R., Dogra, C., Malhotra, S., Sharma, P., & Pal, R. (2006). Diversity, distribution and divergence of *lin* genes in hexachlorocyclohexane-degrading sphingomonads. *Trends in Biotechnology, 24*, 121–130.

Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., et al. (2010). Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *Microbiology and Molecular Biology Reviews, 74*, 58–80.

Luzordo, O. P., Mahtani, V., Troyano, J. M., Alvarez de la Rosa, M., Padilla-Perez, A. I., Zumbado, M., et al. (2009). Determinants of organochlorine levels detectable in the amniotic fluid of women from Tenerif Island (Canary Islands, Spain). *Environmental Research, 109*, 607–613.

Mahmood, A., Malik, R. N., Li, J., & Zhang, G. (2014). Levels, distribution pattern and ecological risk assessment of organochlorines pesticides (OCPs) in water and sediments from two tributaries of the Chenab River, Pakistan. *Ecotoxicology, 23*, 1713–1721.

Manickam, N., Mau, M., & Schlomann, M. (2006). Characterization of the novel HCH degrading strain, *Microbacterium* sp. ITRC1. *Applied Microbiology and Biotechnology, 69*, 580–588.

Manickam, N., Misra, R., & Mayilraj, S. (2007). A novel pathway for the biodegradation of γ-hexachlorocyclohexane by a *Xanthomonas* sp. strain ICH12. *Journal of Applied Microbiology, 102*, 1468–1478.

Manickam, N., Reddy, M. K., Saini, H. S., & Shanker, R. (2008). Isolation of hexachlorocyclohexane-degrading *Sphingomonas* sp. by dehalogenase assay and characterization of genes involved in γ-HCH degradation. *Journal of Applied Microbiology, 104*, 952–960.

Miyauchi, K., Lee, H.-S., Fukuda, M., Takagi, M., & Nagata, Y. (2002). Cloning and characterization of *linR*, involved in regulation of the downstream pathway for γc-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. *Applied and Environment Microbiology, 68*, 1803–1807.

Murthy, R. H. M., & Manonmani, H. K. (2007). Aerobic degradation of technical hexachlorocyclohexane by a defined microbial consortium. *Journal of Hazardous Materials, 149*, 18–25.

Nagata, Y., Endo, R., Itro, M., Ohtsubo, Y., & Tsuda, M. (2007). Aerobic degradation of lindane (γ-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. *Applied Microbiology and Biotechnology, 76*, 741–752.

Nagata, Y., Futamura, A., Miyauchi, K., & Takagi, M. (1999). Two different types of dehalogenases, LinA and LinB, involved in γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26 are localized in the periplasmic space without molecular processing. *Journal of Bacteriology, 17*, 5409–5413.

Nagata, Y., Nariya, T., Ohtomo, R., Fukuda, M., Yano, K., & Takagi, M. (1993). Cloning and sequencing of a dehalogenase gene encoding an enzyme with hydrolase activity involved in the degradation of gamma-hexachlorocyclohexane in *Pseudomonas paucimobilis*. *Journal of Bacteriology, 175*, 6403–6410.

Nagata, Y., Prokop, Z., Sato, Y., Jerabek, P., Kumar, A., Ohtsubo, Y., et al. (2005). Degradation of β-hexachlorocyclohexane by haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. *Applied and Environment Microbiology, 71*, 2183–2185.

Nagpal, V., Srinivasan, M. C., & Paknikar, K. M. (2008). Biodegradation of γ-hexachlorocyclohexane (lindane) by a non-white-rot fungus *Conidiobolus* 03-1-56 isolated from litter. *Indian journal of Microbiology, 48*, 134–141.

Nalin, R., Simonet, P., Vogel, T. M., & Normand, P. (1999). *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. *International Journal of Systematic Bacteriology, 49*, 19–23.

Nitoi, I., Oncescu, T., & Oancea, P. (2013). Mechanism and kinetic study for the degradation of lindane by photo-Fenton process. *Journal of Industrial and Engineering Chemistry, 19*, 305–309.

Okeke, B. C., Siddique, T., Arbestain, M. C., & Frankenberger, W. T. (2002). Biodegradation of γ-hexachlorocyclohexane (lindane) and α-hexachlorocyclohexane in water and a soil slurry by a *Pandoraea* species. *Journal of Agriculture and Food Chemistry, 50*, 2548–2555.

Ondarza, P. M., González, M., Fillmann, G., & Miglioranza, K. S. B. (2014). PBDEs, PCBs and organochlorine pesticides distribution in edible fish from Negro River basin, Argentinean Patagonia. *Chemosphere, 94*, 135–142.

Pesce, S. F., Cazenave, J., Monferrán, M. V., Frede, S., & Wunderlin, D. A. (2008). Integratedsurvey on toxic effects of lindane on neotropical fish: *Corydoras paleatus* and *Jenynsia multidentata*. *Environmental Pollution, 156*, 775–783.

Pesce, S. F., & Wunderlin, D. A. (2004). Biodegradation of lindane by a native bacterial consortium isolated from contaminated river sediment. *International Biodeterioration and Biodegradation, 54*, 255–260.

Phillips, T. M., Seech, A. G., Lee, H., & Trevors, J. T. (2005). Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. *Biodegradation, 16*, 363–392.

Pino, N. J., Domínguez, M. C., & Penuela, G. A. (2011). Isolation of a selected microbial consortium capable of degrading methyl parathion and p-nitrophenol from a contaminated soil site. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 46*, 173–180.

Polti, M. A., Aparicio, J. D., Benimeli, C. S., & Amoroso, M. J. (2014). Simultaneous bioremediation of Cr(VI) and lindane in soil by actinobacteria. *International Biodeterioration and Biodegradation, 88*, 48–55.

Quintero, J. C., Lu-Chau, T. A., Moreira, M. T., Feijoo, G., & Lema, J. M. (2007). Bioremediation of HCH present in soil by the white-rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. *International Biodeterioration and Biodegradation, 60*, 319–326.

Quintero, J. C., Moreira, M. T., Feijoo, G., & Lema, J. M. (2005). Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. *Chemosphere, 61*, 528–536.

Quintero, J. C., Moreira, M. T., Lema, J. M., & Feijoo, G. (2006). An anaerobic bioreactor allows the efficient degradation of HCH isomers in soil slurry. *Chemosphere, 63*, 1005–1013.

Rigas, F., Papadopoulou, K., Philippoussis, A., Papadopoulou, M., & Chatzipavlidis, J. (2009). Bioremediation of lindane contaminated soil by *Pleurotus ostreatus* in non sterile conditions using multilevel factorial design. *Water, Air, and Soil pollution, 197*, 121–129.

Roche, H., Vollaire, Y., Persic, A., Buet, A., Oliveira-Ribeiro, C., Coulet, E., et al. (2009). Organochlorines in the Vaccarès Lagoon trophic web (Biosphere Reserve of Camargue, France). *Environmental Pollution, 157*, 2493–2506.

Roy, C., Gaillardon, P., & Montfort, F. (2000). The effect of soil moisture content on the sorption of five sterol biosynthesis inhibiting fungicides as a function of their physicochemical properties. *Pest Management Science, 56*, 795–803.

Saadati, N., Abdullah, M. P., Zakaria, Z., Rezayi, M., & Hosseinizare, N. (2012). Distribution and fate of HCH isomers and DDT metabolites in a tropical environment—case study Cameron Highlands-Malaysia. *Chemistry Central Journal, 6*, 130.

Saez, J. M., Alvarez, A., Benimeli, C. S., & Amoroso, M. J. (2014). Enhanced lindane removal from soil slurry by immobilized *Streptomyces* consortium. *International Biodeterioration and Biodegradation, 93*, 63–69.

Sagar, V., & Singh, D. P. (2011). Biodegradation of lindane pesticide by non white-rots soil fungus *Fusarium* sp. *World Journal of Microbiology and Biotechnology, 27*, 1747–1754.

Salam, J. A., & Das, N. (2012). Remediation of lindane from environment-an overview. *International Journal of Advanced Biological Research, 2*, 9–15.

Salam, J. A., & Das, N. (2014). Lindane degradation by *Candida* VITJzN04, a newly isolated yeast strain from contaminated soil: Kinetic study, enzyme analysis and biodegradation pathway. *World Journal of Microbiology and Biotechnology, 30*, 1301–1313.

Salam, J. A., Lakshmi, V., Das, D., & Das, N. (2013). Biodegradation of lindane using a novel yeast strain, *Rhodotorula* sp. VITJzN03 isolated from agricultural soil. *World Journal of Microbiology and Biotechnology, 29*, 475–487.

Santoyo Telope, F., (2009) The central role of *Burkholderia* sp. in the degradation of 2,4-D by a mixed culture immobilized on a fixed bed reactor (in Spanish). Ph.D. thesis, National Polytechnic Institute, National School of Biological Sciences, Mexico.

Shaker, E. M., & Elsharkawy, E. E. (2015). Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agro industrial areas in Assiut, Egypt. *Environmental Toxicology and Pharmacology, 39*, 433–440.

Shong, J., Jimenez Diaz, M. R., & Collins, C. H. (2012). Towards synthetic microbialconsortia for bioprocessing. *Current Opinion in Biotechnology, 23*, 798–802.

Tor, A., Aydin, M. E., Aydin, S., Tabakci, M., & Beduk, F. (2013). Removal of lindane from an aqueous solution by using aminopropylsilica gel-immobilized calix[6]arene. *Journal of Hazardous Materials, 262*, 656–663.

Ulčnik, A., Kralj Cigić, I., Zupančič-Kralj, L., Tavzes, Č., & Pohleven, F. (2012). Bioremediation of lindane by wood-decaying fungi. *Drvna Industrija, 63*, 271–276.

Vijgen, J. (2006) The legacy of lindane HCH isomer production: A global overview of residue management, formulation and disposal. International HCH and Pesticide Association. http://www.ihpa.info/library_access.php

Vijgen, J., Abhilash, P. C., Li, Y. F., Lal, R., Forter, M., Torres, J., et al. (2011). Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs—A global perspective on the management of lindane and its waste isomers. *Environmental Science and Pollution Research, 18*, 152–162.

Villaamil Lepori, E. C., Bovi Mitre, G., & Nassetta, M. (2013). Situación actual de la contaminación por plaguicidas en Argentina. *Revista Internacional de Contaminación Ambiental, 29*, 25–43.

Vlčková, K., & Hofman, J. (2012). A comparison of POPs bioaccumulation in *Eisenia fetida* in natural and artificial soils and the effects of aging. *Environmental Pollution, 160*, 49–56.

Wu, J., Hong, Q., Sun, Y., Hong, Y., Yan, Q., & Li, S. (2007). Analysis of the role of LinA and LinB in biodegradation of δ-hexachlorocyclohexane. *Environmental Microbiology, 9*, 2331–2340.

Zhang, H., Hu, C., Jia, X., Xu, Y., Wu, C., Chen, L., et al. (2012). Characteristics of γ-hexachlorocyclohexane biodegradation by a nitrogen-fixing cyanobacterium, *Anabaena azotica*. *Journal of Applied Phycology, 24*, 221–225.

Zheng, G., Selvam, A., & Wong, J. W. C. (2011). Rapid degradation of lindane (γ-hexachlorocyclohexane) at low temperature by *Sphingobium* strains. *International Biodeterioration and Biodegradation, 65*, 612–618.

Degradation of Atrazine by Plants and Microbes

S.N. Singh and Nitanshi Jauhari

1 Introduction

Application of herbicides (or pesticides) is inevitable in the management practices of crop production in modern agriculture. Synthetic chemicals are designed to target eradication of weeds through specific mechanisms to minimize their competition with crop plants for use of nutrients and water from agricultural fields. Despite of all precautions, herbicides also adversely affect crops, microbes and animals. The use of herbicides not only affects the crop growth and development, but also the quality of crops. Thus, the herbicide pollutes the food chain, threatening the human health at large. Hence, there is an impending need to develop strategies to minimize the soil pesticide residues, water contamination and the toxicity of herbicides.

Among several chemical herbicides, atrazine (2-chloro-4-ethyl amino-y-6-isopropylamino-s-triazine, $C_8H_{14}ClN_5$) is one of the most widely used herbicides to eradicate many weeds in order to enhance crop production. The chemical structure of atrazine has been shown in Fig. 1.

It was discovered in 1950s and became most popular due to its high effectiveness against a wide spectrum of weeds. It is moderately soluble in water (33 mg L^{-1} at 22 °C) (Tomlin 2000). An ideal herbicide should kill only targeted weeds and be easily biodegradable. Neither it should leach to the groundwater nor spread in the surroundings. However, this is likely to be rare quality of an herbicide or pesticide. Atrazine is rapidly degraded in soil with a half-life of 32–128 days (Krutz et al. 2008). However, its half-life becomes longer in sub-surface environments. Atrazine degradation varies from rapid (half-life 38 days) to no degradation in groundwater. However, atrazine half-life varied between 430 and 829 days in the anaerobic

S.N. Singh (✉) · N. Jauhari
Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow 226001, India
e-mail: drsn06@gmail.com

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5_10

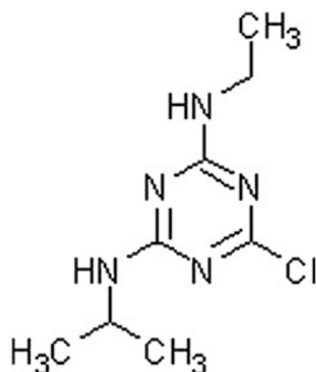


Fig. 1 Chemical structure of atrazine (formula: $C_8H_{14}ClN_5$)

conditions (Talja et al. 2008). It has been recognized as an endocrine disrupting agent and moderately toxic to humans and animals. This is the reason that use of atrazine was banned in 1992 by the European Union for its persistence in groundwater. However, it is widely used as a herbicide in other countries outside the European Union, such as Brazil, China, India and Russia. About 3.4×10^4 tons of atrazine is applied in USA every year (Sadler et al. 2014), while China has usage of atrazine as much as 10820 tons in 2020. In Finland, Vuorimaa et al. (2007) found atrazine and its metabolites e.g. desethylatrazine (DEA), deisopropylatrazine (DIA) and desethyldeisopropylatrazine (DEDIA) in groundwater.

Atrazine is highly persistent in soil and gets accumulated over the period, as its microbial degradation rate is very slow as compared to its application rate (Fuscaldo et al. 1999). As a consequence, it becomes a serious threat to soil health which hampers plant growth and development. Further, atrazine moves to water sources through migration and leaching and thus becomes a potential threat to both wildlife and human beings. As of today, atrazine and its metabolites have been reported to be an alarming threat to ground and surface water exceeding threshold of 3 $\mu g\ L^{-1}$ as decided by US EPA. However, World Health Organization (WHO) restricted the permissible limit to only 2 $\mu g\ L^{-1}$. Thus, atrazine is a cause for high environmental threat due to its low biodegradability and its potential to contaminate both surface and ground water (Chan and Chu 2007).

Among the pesticides, atrazine is one of the most widely used herbicides throughout the world. It is extensively used on crops, like sugarcane, corn, sorghum, pineapple, conifers, forestry, grassland, macadamia nuts etc. (Luciane et al. 2010).

Atrazine (2 chloro-4 ethylamine-6-isopropyamine-5-triazine) is a chlorinated systemic selective herbicide used widely to eradicate weeds globally (Fig. 1). Atrazine is highly persistent in soil with average half-life ranging between 13 and 261 days (US EPA 2003). However, in river water, atrazine can persist for more than 100 days (Seiler et al. 1992). However, in sea water, it can stay for only 10 days (Armbrust and Crosby 1991) and about 660 days in the anaerobic conditions. No degradation was observed after incubation with adapted activated sludge.

Thus, due to its high mobility and persistence, residence of atrazine and its derivatives were detected in soil, surface water and groundwater after year's application (Schiavon 1988). Atrazine even at ppb level disrupts sexual development in amphibians, thus posing a serious ecological risk (Rhine et al. 2003).

Although many countries avoided using of atrazine due to its high toxicity, but it is still most popular herbicide in many countries (Jin and Ke 2002). In India, atrazine is still extensively used; hence there is high possibility of contamination of soil and water with atrazine in many parts. Therefore, there is a need to develop effective clean up technology for removal of atrazine. Due to its low biodegradability and high toxicity, it is still an environmental threat and has potential to contaminate surface and ground water (Chan and Chu 2007).

2 Atrazine Degradation

2.1 Physico-Chemical Degradation

Several physico-chemical techniques are in place for cleanup of atrazine from water, wastewater and contaminated soils, e.g. incineration, thermal absorption, UV, peroxides, metal oxides, reverse osmosis and electrodylysis (Rodrigo et al. 2014). These technologies are generally expensive and also cause formation of toxic by-products. In some cases, end products need to be further treated.

The natural dissipation of atrazine from soil is regulated by both biotic and abiotic processes. The clay and organic matter of the soil determine the extent of adsorption of atrazine to particles of soil and sediment (Nemeth-Konda et al. 2002). Adsorption of atrazine increases the accumulation of atrazine by reducing its availability. In this situation, soil microbes play an important role in the degradation of atrazine. Biodegradation of atrazine in soil is more in surface soil than in sub-surface zone. Low temperature and lack of degrading microbes are primary factors which limit atrazine degradation in the sub-surface aquifer conditions and vadose zone (Radosevich et al. 1989). Atrazine was considered to be moderately persistent in soil. However, in past several years, many bacterial strains were isolated which could completely mineralize atrazine (Sadowsky and Wackett 2001).

2.2 Degradation of Atrazine by Plants

A few plants, which were found tolerant to organic xenobiotics, could remove pesticides by taking up through roots and degrading them via putative metabolic pathways (Schröder et al. 2002; Cui and Yang 2011; Zhang et al. 2011). Besides, some plants could clean up xenobiotics in conjugation with microbes (Jin et al. 2012; Li and Yang 2013). However, in this case, microbes play an important role in

degradation of herbicides, as rapid degradation of herbicides was caused by rhizospheric bacteria through specific enzymes (Tesar et al. 2002; Merini et al. 2009; Jin et al. 2012). However, for faster degradation of toxic compounds, special plant species have to be selected or genetically engineered (Richard 2000). Several dicots and monocots show high tolerance to toxic herbicides and some genotypes of these plants were found to degrade herbicides in the soil. Usually plant species with fibrous root system have high ability to absorb the pollutants through broad root surface and interact with soil microbes for biodegradation of herbicides. These microbes thrive mainly on the root exudates.

Lolium multiflorum is a plant species which thrives well even in the adverse environmental conditions. However, the process of atrazine accumulation and degradation mediated by ryegrass is yet not fully understood. Sui and Yang (2013) studied the accumulation and degradation of atrazine in several rye grass genotypes. Out of these, three genotypes of rye grass had potential to accumulate and degrade atrazine. They could translocate the root-mediated uptake of atrazine to above ground. Thus, these genotypes may be used for the phytoremediation of atrazine—contaminated soil on a large scale.

Plant root exudates also influence the degradation of atrazine through their impact on microbial activity. Fang et al. (2001) observed faster degradation of atrazine in contaminated soil planted with *Pennisetum clandestinum* than in unplanted soil (Singh et al. 2004a, b).

2.3 Bacterial Degradation

Many other bacteria are currently known to degrade atrazine which include the members of different genera, such as *Acinetobacter* (Singh et al. 2004a, b), *Agrobacterium* (Struthers et al. 1998), *Arthobacter* (Zhang et al. 2011), *Chelatobacter* (Cheyns et al. 2012), *Dehftia* (Vargha et al. 2005).

Many bacteria were isolated and studied for degradation of atrazine by members of genera *Pseudomonas, Acinetobacter, Agrobacterium, Arthrobacter, Rastonia* and *Norcardioides* etc. (Bouquard et al. 1997a, b; Struthers et al. 1998; Strong et al. 2002).

Atrazine biodegradation occurs via different pathways that funnel into cyanuric acid metabolism. The gene region of *Pseudomonas* sp. strain ADP encoding atrazine degradation enzymes has been cloned and characterized. Initially, atrazine is degraded to hydroxyatrazine by hydrolytic dechlorination (de Souza et al. 1995). Later on, two enzymes of aminohydrolase protein catalyzed the sequential removal of ethylamine and isopropylamine (Boundy-Mills et al. 1997; Sadowski et al. 1998).

Nocardioides (Omotayo et al. 2012), *Pseudaminobacter* (Topp et al. 2000), *Pseudomonas* (Hernandez et al. 2008), *Rastonia* (Stamper et al. 2002), *Rhizobium* (Bouquard et al. 1997a, b) and *Rodococcus* (Behki et al. 1993) have been reported to degrade atrazine. Among these bacteria, *Pseudomonas* sp. strain ADP reported

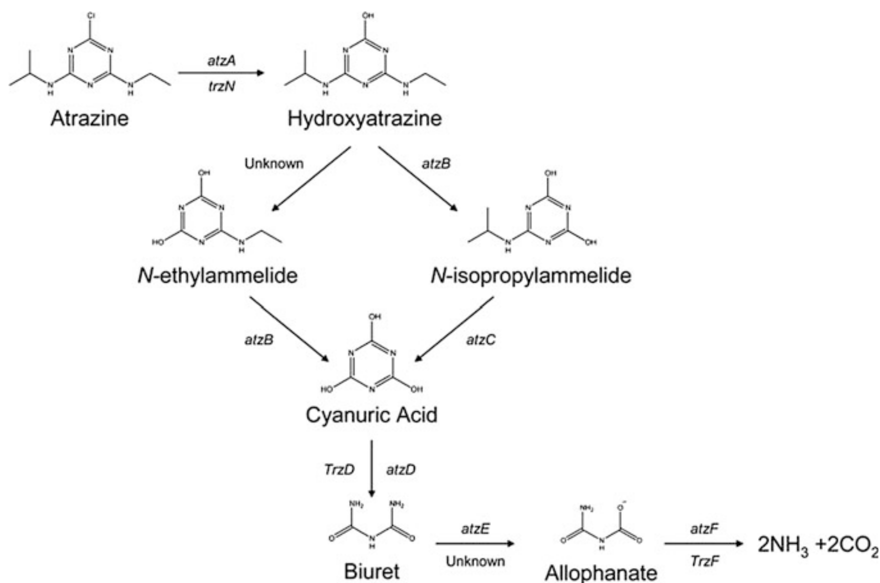


Fig. 2 Atrazine degradation by bacteria (Krutz et al. 2008)

by Mandelbaum et al. (1995), was used to elucidate the sequences of catabolic enzymes involved in aerobic degradation pathway and to develop probes for the genes encoding these enzymes. Krutz et al. (2008) elucidated the bacterial metabolic pathway of atrazine degradation (Fig. 2).

They suggested that a unique operon of genes encoding s-triazine degradation was evolved in areas in which this herbicide was extensively applied. The gene regions encoding the first three enzymes responsible for atrazine degradation have been isolated and characterized from *Pseudomonas* sp. strain ADP (Boundy-Mills et al. 1997; de Souza et al. 1995, 1996; Sadowski et al. 1998). This bacterium could mineralize a very high concentration of 500 mg L^{-1} under both growth and non-growth conditions, using the atrazine as the sole nitrogen source (Mandelbaum et al. 1995). The atzA gene, which encodes atrazine chlorohydrolase, dechlorinates atrazine to nonphytotoxic metabolite hydroxy atrazine (Fig. 3). The next step in the degradation pathway is hydrolytic removal of aminoethyl group from hydroxyatrazine by the hydroxyatrazine ethyl amidohydrolase which is *atz*B gene product. Finally, the *atz*C gene encodes for another aminohydrolase that converts N-isopropylammelide to cyanuric acid. Besides, Martinez et al. (2001) have sequenced the complete catabolic plasmid from *Pseudomonas* sp. strain ADP and identified three additional genes *atz*D, *atz*E and *atz*F encoding for cyanuric acid amidohydrolase, biurel-hydrolase and allophanate hydrolase. Thus, total genetic basis for complete atrazine metabolism in *Pseudomonas* sp. strain ADP was worked out.

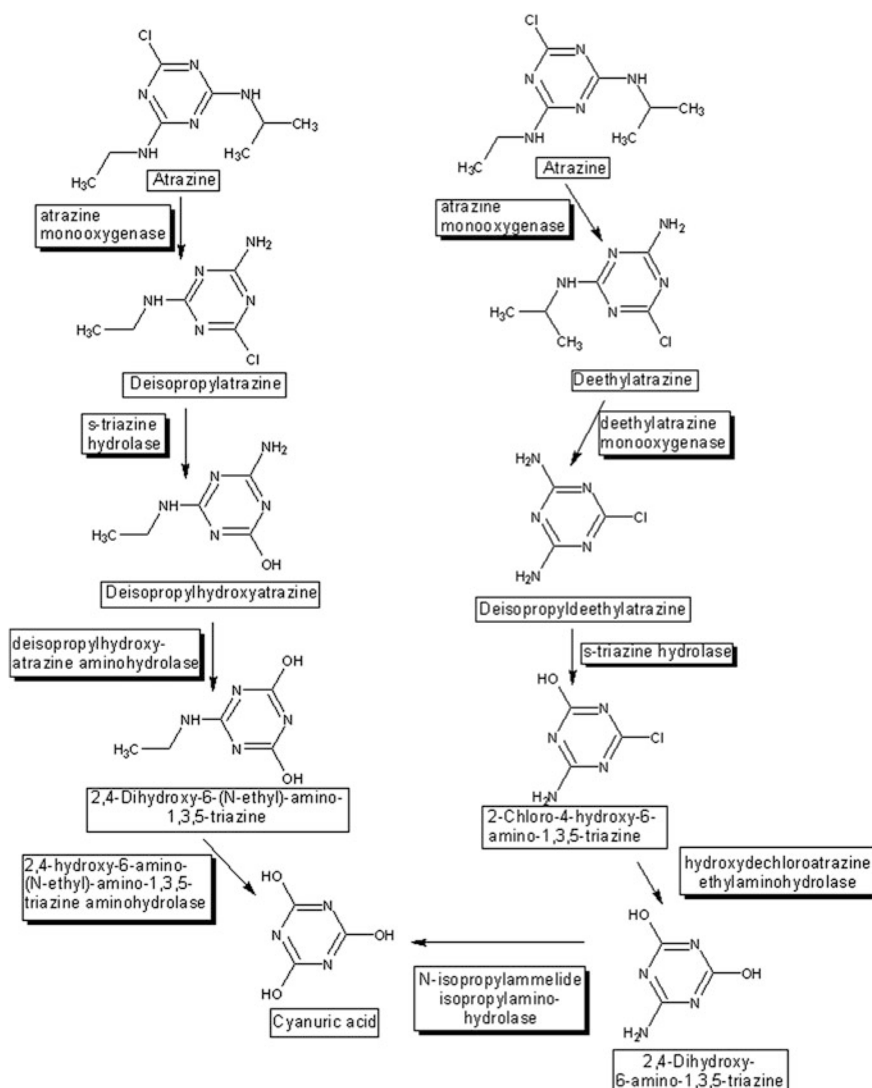


Fig. 3 Bacterial degradation pathway of atrazine (Wackett et al. 2002)

Degradation of atrazine occurs through different pathways. One pathway involves dealkylation of amino groups to give 2-chloro-4-hydroxy-6-amino-1,3,5-triazine. The another pathway, which is well established, involves hydrolysis of C-C_1 bond, ethyl and isopropyl groups leading to formation of cyanuric acid which is further utilized to release CO_2 (Sagarkar et al. 2013). Similar catabolic pathway of atrazine degradation was reported by Wackett et al. (2002) and Fazlurrahman et al. (2009). Surprisingly, there was no difference in the

intermediates formed during atrazine degradation in the presence and absence of surfactants like rhamnolipids and triton X-100. However, addition of surfactant in soil enhanced the degradation of atrazine by increasing desorption of atrazine from soil and making it to more available for degradation by bacteria. However, glycolipid-type surfactant like rhammolipids exhibited higher potential of enhanced degradation than synthetic surfactant like triton X-100.

The characterization of atrazine-degrading bacterial strains revealed the presence of *atz*ABCDEF genes in plasmid and encoding enzymes were found in the oxygen-dependent degradation of atrazine (de Souza et al. 1996; Martinez et al. 2001). Further, investigation revealed that genes are highly conserved in different microbial genera. Their worldwide spread indicates a potential molecular mechanism for the dispersion of the *atz*ABC genes to other soil bacteria (de Souza et al. 1998). Besides, two other atrazine-degrading genes encoding the enzymes atrazine chlorohydrolase and cyanuric acid hydrolase, were characterized as *trz*N and *trz*D, respectively (Karns 1999; Mulbry et al. 2002).

2.3.1 Atrazine Degradation by Microbial Consortia

Microbial degradation of atrazine follows different metabolic pathways, involving stepwise transformations carried out by individual species or microbial consortia. For this purpose, several atrazine degrading bacteria were isolated and their separate metabolic pathways for atrazine degradation were thoroughly studied, but their synergistic action in atrazine metabolism is still not well investigated.

In a study of atrazine degradation by microbial consortia, it was first observed that atrazine was dechlorinated to hydroxyatrazine in two ways. In one pathway, hydroxyatrazine was transformed by *Nocardia* sp. to N-ethylammelide via unidentified product, whereas in another pathway, hydroxyatrazine was hydrolyzed to N-isopropylammelide by *Rhizobium* sp. having the gene *atz*B. However, all the consortium members contained *atz*C responsible for cleavage of the ring, besides the gene *trz*D. However, Smith et al. (2005) reported that none of the microbe carried all three genes i.e. *atz*D, *atz*E, and *atz*F.

Subsequently, Kolic et al. (2007) found that a four member microbial community enriched from an agrochemical factory was able to mineralize atrazine very rapidly to CO_2 up to 78 % within a week. When the genetic potential of community members was studied individually, it was observed that *Arthrobacter* strain ATZ1 with *trz*N and *atz*C genes and ATZ2 strain having *trz*N, *atz*B and *atz*C genes, could be involved in the upper pathway, producing cyanuric acid, while others *Ochrobacterium* sp. CA1 and *Pseudomonas* sp. CA2, both with *trz*D gene, were found involved in cyanuric acid metabolism.

Microbial community members in biofilms interact at the genetic level among intra and inter species and are also exposed to genetic events like transformation, transduction and conjugation at the contaminated site. Hence, they acquired improved capability to degrade hazardous substances (Stoodley et al. 2002). It was

observed that microbes modified the biofilms through natural transformation to improve their degradation ability (Perumbakkam et al. 2006). They transformed biofilm communities with the gene *atzA* which encoded atrazine chlorohydrolase. Both pure and soil-borne culture may be transferred with *atzA* gene cloned in plasmid PBBR1NCSS, to have the ability to degrade atrazine. Thus natural transformation may be used as a tool to enhance atrazine biodegradation performed by biofilms.

2.4 Fungal Degradation of Atrazine

Several fungi like *Aspergillus fumigates, A. flavipes, Fusarium moniliforme*, *Penicillium decumbens*, *Rhizopus stolonifer, Trichoderma viride* etc. are reported to degrade atrazine (Kaufman and Blake 1970; Mougin et al. 1994). Fungal degradation begins with N-dealkylation leading to formation of deethylatrazine and deisopropylatraine as the degradation products (Kaufman and Blake 1970). A white rot fungus i.e. *Phanerochaete chrysosporium*, could metabolize 48 % of atrazine in the growth medium after 4 days of incubation. In this process, 25 % of mineralization of ethyl group of herbicide occurred with the formation of hydroxylated and/or N-dealkylated metabolites. However, mineralization of 14C-labeled atrazine was not reported (Mougin et al. 1994). Besides, *Pleurotus pulmonarius* is another fungus which degraded atrazine in liquid medium and formed N-dealkylated metabolites, such as deethylatrazine, deisopropylatrazine, deethyliecthyatrazine and also hydroxyisopropyl atrazine as the hydroxypropyl metabolites (Masaphy et al. 1996a). Atrazine transformation was enhanced in the presence of 300 μM of maganese by this fungus with the accumulation of both N-dealkylated and propylhydroxylated metabolites (Masaphy et al. 1996b).

2.5 Strategies for Enhanced Atrazine Degradation by Microbes

In addition to natural occurring microbes, genetically engineered microbes have been used for boost up degradation of xerobiotic compounds. As reported by Rousseaux et al. (2001), *Chelatobacter heintzii* Cit1 could mineralize atrazine in soil 3 folds more with the inoculum of 10^4 UFCg^{-1} than the control. Gupta and Baummer (1996) investigated the effect of poultry on the biodegradation of atrazine and found that atrazine removal was enhanced by 2 folds as compared to soil without poultry manure. However, mineral nutrients, like nitrates and phosphates, played no role in atrazine degradation.

In a field study of soil contaminated with atrazine spillover (approx. 29000 ppm), bioaugmentation was performed using a killed and stabilized whole

cell suspension of recombinant *E. coli* to overproduce atrazine chlorohydralase, *atz*A. It was observed that there was a decline of 52 % in atrazine level in plots having killed recombinant *E. coli* cells. In another investigation, cell-free crude extracts from *Pseudomonas* sp. ADP containing the enzymes that catalyzed atrazine degradation, were entrapped in sol-gel glass. In this situation, there was a significant loss of enzyme activity as compared to non-entrapped crude extract (Kauffmann and Mandelbaum 1996). This strategy seems to be more promising than use of transgenic bacterial cells to field application. Silva et al. (2004) observed that bioaugmentation of *Pseudomonas* sp. ADP in combination with biostimulation with citrate or succinate, markedly enhanced atrazine mineralization. In comparison to other carbon sources, cellulose enhanced dealkylation of atrazine side-chain by soil microbes as compared to other carbon substrates. Glucose, as an end-product of cellulose depolymerization, may be responsible of inhibition of dealkylaion enzyme, resulting in a decrease in atrazine side chain mineralization (Yassir et al. 1998). Atrazine degradation by an anaerobic mixed culture was found higher in co-metabolic process than in absence of external C and N sources.

Most of atrazine degradation bacteria use herbicides as a source of nitrogen. Hence, presence of another N source decreased atrazine degradation by *Pseudomonas* sp. ADP (Clausen et al. 2002). Nitrogen is found detrimental to atrazine degradation, but stimulated the primary growth of bacteria. Thus microbial processes and C uptake used to influence the herbicide degradation.

3 Effect of Atrazine on Microbial Communities

Microbes play an important role in the functions of natural ecosystems, such as organic matter decomposition, nutrient cycling and natural attenuation of toxic compounds. Thus, they provide invaluable service to soil and water purification processes by their metabolic activities.

Abiotic and biotic factors both influence the structural composition and diversity of microbial community. These factors may be classified as natural and anthropogenic factors. The natural factors are vegetation, temperature, moisture, pH of the soil, while anthropogenic activities are management of soil through tillage and application of pesticides and fertilizers (Zhou et al. 2008). Soil microbes are adversely affected by the application of pesticides including fungicides, insecticides and herbicides.

Hu et al. (2005) studied the effect of atrazine application at the rate of 50 mg kg^{-1} to soil and found that soil respiration rate was enhanced, but microbial diversity was reduced. Atrazine application significantly shifted the microbial community structure and function (Seghers et al. 2003). Besides, Chen et al. (2015) found an inhibitory effect on the soil nitrification process and microbial community in microcosm incubation.

4 Conclusion

Microbial degradation of atrazine is an eco-friendly and low cost technology. Better understanding of microbial mineralization of atrazine by indigenous or transgenic microbes can boost up elimination of atrazine from agricultural soils in a cost-effective manner. Microbes have the inherent ability to utilize herbicides as carbon and nitrogen sources. Our understanding about atrazine degradation can lead to development of an effective bioremediation process for other recalcitrant herbicides, used in agriculture.

References

Armbrust, D. L., & Crosby, D. G. (1991). Fate of carbaryl 1-naphthol and atrazine in seawater. *Pacific Science, 45*, 314–320.

Behki, R., Topp, E., Dick, W., & Germon, P. (1993). Metabolism of the herbicide atrazine by *Rhodococcus* strains. *Applied and Environment Microbiology, 59*, 1955–1959.

Boundy-Mills, K. L., de Souza, M. L., Mandelbaum, R. T., Wackett, L. P., & Sadowsky, M. J. (1997). The *atz*B gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Applied and Environment Microbiology, 63*, 916–923.

Bouquard, C., Ouazzani, J., Prome, J., Michel-Briand, Y., & Plesiat, P. (1997a). Dechlorination of atrazine by a *Rhizobium* sp. Isolate. *Applied and Environment Microbiology, 63*, 862–866.

Bouquard, C., Ouzzani, J., Prome, J. C., Michel Briand, Y., & Plesiat, P. (1997b). Dechlorination of atrazine by a *Rhizobium* isolate. *Applied and Environment Microbiology, 63*, 862–866.

Chan, K. H., & Chu, W. (2007). Effect of humic acid on the photolysis of the pesticide atrazine in a surfactant-aided soil-washing system in acidic condition. *Water Research, 39*, 2154–2166.

Chen, Q., Yang, B., Wang, H., He, F., Gao, Y., & Scheel, R. (2015). Soil microbial community toxic response to atrazine and its residues under atrazine and lead contamination. *Environmental Science and Pollution Research, 22*, 996–1007.

Cheyns, K., Calcoen, J., Martin-Laurent, F., Bru, D., Smolders, E., & Springael, D. (2012). Effects of dissolved organic matter (DOM) at environmentally relevant carbon concentrations on atrazine degradation by *Chelatobacter heintzii* SalB. *Applied Microbiology and Biotechnology, 95*, 1333–1341.

Clausen, G. B., Larsen, L., Johnsen, K., de Lipthay, J. R., & Aamand, J. (2002). Quantification of the atrazine degrading *Pseudomonas* sp. strain ADP in aquifer sediment by quantitative competitive polymerase chain reaction. *FEMS Microbiology Ecology, 41*, 211–229.

Cui, L. E., & Yang, H. (2011). Accumulation and residue of napropamide in alfalfa (*Medicago sativa*) and soil involved in toxic response. *Journal of Hazardous Materials, 190*, 81–86.

De Souza, M. L., Newcombe, D., Alvey, S., Crowley, D. E., Hay, A., Sadowsky, M. J., et al. (1998). Molecular basis of a bacterial consortium interspecies catabolism of atrazine. *Applied and Environment Microbiology, 64*, 178–184.

De Souza, M. L., Sadowsky, M. J., & Wackett, L. P. (1996). Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: Gene sequence, enzyme purification, and protein characterization. *Journal of Bacteriology, 178*, 4894–4900.

De Souza, M. L., Wackett, L. P., Boundy-Mills, K. L., Mandelbaum, R. T., & Sadowsky, M. J. (1995). Cloning, characterization, and expression of a gene region from *Pseudomonas* sp. strain ADP involved in the dechlorination of atrazine. *Applied and Environment Microbiology, 61*, 3373–3378.

Fang, C., Radosevich, M., & Fuhrmann, J. J. (2001). Atrazine and phenanthrene degradation in grass rhizosphere soil. *Soil Biology and Biochemistry, 3*, 671–678.

Fazlurrahman, B. M., Pandey, J., Suri, C. R., & Jain, R. K. (2009). Isolation and characterization of an atrazine-degrading Rhodococcussp. strain MB-P1 from contaminated soil. *Letters in Applied Microbiology, 49*, 721–729.

Fuscaldo, F., Bedmar, F., & Monterubbianesi, G. (1999). Persistence of atrazine, metribuzin and simazine herbicides in two soils. *Pesquisa Agropecuária Brasileira, 34*, 2037–2044.

Gupta, G., & Baummer, J, I. I. I. (1996). Biodegradation of atrazine in soil using poultry litter. *Journal of Hazardous Materials, 45*, 185–192.

Hernandez, M., Morgante, V., Avila, M., Villalobos, P., Miralles, P., Gonzalez, M., & Seeger, M. (2008). Novel s-triazine-degrading bacteria isolated from agricultural soils of central Chile for herbicide bioremediation. *Electronic Journal of Biotechnology 11*(5 Special Issue), 1–7.

Hu, J., Dai, X. Z., & Li, S. P. (2005). Effects of atrazine and its degrader *Exiguobaterium* sp. BTAH1 on soil microbial community. *Chinese Journal of Applied Ecology, 8*, 138–143.

Jin, R., & Ke, J. (2002). Impact of atrazine disposal on the water resources of the Yang river in Zhangjiakou area in China. *Bulletin of Environment Contamination and Toxicology, 68*, 893–900.

Jin, Z. P., Luo, K., Zhang, S., Zheng, Q., & Yang, H. (2012). Bioaccumulation and catabolism of prometryne in green algae. *Chemosphere, 87*, 278–284.

Karns, J. S. (1999). Gene sequence and properties of an s-triazine ring-cleavage enzyme from *Pseudomonas* sp. strain NRRLB-12227. *Applied and Environment Microbiology, 65*, 3512–3517.

Kauffmann, C. G., & Mandelbaum, R. T. (1996). Entrapment of atrazine-degrading enzymes in sol-gel glass. *Journal of Biotechnology, 51*, 219–225.

Kaufman, D. D., & Blake, J. (1970). *Soil Biology and Biochemistry, 2*, 73–80.

Kolic, N. U., Hrsak, D., Kolar, A. B., Petric, I., Stipicevic, S., Soulas, G., et al. (2007). Combined metabolic activity with an atrazine-mineralizing community enriched from agrochemical factory soil. *International Biodeterioration and Biodegradation, 60*, 299–307.

Krutz, L. J., Shaner, D. L., Accinelli, C., Zablotowicz, R. M., & Henry, W. B. (2008). Atrazine dissipation in-triazine–adapted and nonadapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters. *Journal of Environmental Quality, 37*, 848–857.

Li, Y. Y., & Yang, H. (2013). Bioaccumulation and degradation of pentachloronitrobenzene in *Medicago sativa*. *Journal of Environmental Management, 119*, 143–150.

Luciane, S., Attilio, C., Geslaine, A. R. S., & Rita, D. C. G. S. (2010). New aspects on atrazine biodegradation. *Brazilian Archives of Biology and Technology, 53*, 487–496.

Mandelbaum, R. T., Allan, D. L., & Wackett, L. P. (1995). Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. *Applied and Environment Microbiology, 61*, 1451–1457.

Martinez, B., Tomkins, J., Wackett, R., Wing, R., & Sadowsky, M. J. (2001). Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *Journal of Bacteriology, 183*, 5684–5697.

Masaphy, S., Henis, Y., & Levanon, D. (1996a). Manganese-enhanced biotransformation of atrazine by the white rot fungus *Pleurotus pulmonarius* and its correlation with oxidation activity. *Applied and Environment Microbiology, 2*, 3587–3593.

Masaphy, S., Levanon, D., & Henis, Y. (1996b). Degradation of atrazine by the lignocellulolytic fungus *Pleurotus pulmonarius* during solid—state fermentation. *Bioresource Technology, 56*, 207–214.

Merini, L. J., Bobillo, C., Cuadrado, V., Corach, D., & Giulietti, A. M. (2009). Phytoremediation potential of the novel atrazine tolerant *Lolium multiflorum* and studies on the mechanisms involved. *Environmental Pollution, 157*, 3059–3063.

Mougin, C., Laugero, C., Asther, M., Dubroca, J., Frasse, P., & Asther, M. (1994). Biotransformation of the herbicide atrazine by the white rot fungus *Phanerochaete chrysosporium*. *Applied and Environment Microbiology, 60*, 705–708.

Mulbry, W. W., Zhu, H., Nour, S. M., & Topp, E. (2002). The triazine hydrolase gene *trz*N from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. *FEMS Microbiology Letters, 206*, 75–79.

Nemeth-Konda, L., Füleky, G., Morovjan, G., & Csokan, P. (2002). Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil. *Chemosphere, 48*, 545–552.

Omotayo, A. E., Ilori, M. O., Radosevich, M., & Amund, O. O. (2012). Metabolism of atrazine in liquid cultures and soil microcosms by Nocardioides strains isolated from a contaminated nigerian agricultural soil. *Soil and Sediment Contamination: An International Journal, 22*, 365–375.

Perumbakkam, S., Hess, T. F., & Crawford, R. L. (2006). A bioremediation approach using natural transformation in pure-culture and mixed-population biofilms. *Biodegradation, 17*, 545–557.

Radosevich, M., Traina, S. J., & Tuovinen, O. H. (1989). Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. *Biodegradatin, 7*, 137–149.

Rhine, E. D., Fuhrmann, J. J., & Radisevich, M. (2003). Microbial community response to atrazine exposure and nutrient availability: Linking degradation capacity to community structure. *Microbial Ecology, 46*, 145–160.

Richard, B. M. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology, 3*, 153–162.

Rodrigo, M. A., Oturan, N., & Oturan, M. A. (2014). Electrochemically assisted remediation of pesticides in soils and water: A review. *Chemical Reviews, 114*, 8720–8745.

Rousseaux, S., Hartmann, A., & Soulas, G. (2001). Isolation and characterisation of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. *FEMS Microbiology Ecology, 36*, 211–222.

Sadler, E. J., Sudduth, K. A., Lerch, R. N., Baffaut, C., & Kitchen, N. R. (2014). A simple index explains annual atrazine transport from surface runoff-prone watersheds in the north-central USA. *Hydrological Processes, 28*, 210–217.

Sadowski, M. J., Tong, Z., Souza, M. L. D., & Wackett, L. P. (1998). AtzC is a new member of the aminohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. *Journal of Bacteriology, 180*, 152–158.

Sadowsky, M. J., & Wackett, L. P. (2001). Genetics of atrazine ands-triazine degradation by *Psedomonas* sp. strain ADP and other bacteria. In J. C. Hall, R. E. Hoagland & R. M. Zablotowicz (Eds.), *Pesticide biotransformation in plants and microorganisms: Similarities and divergences*. ACS Symposium Series 777 (pp. 268–282). Washington, DC: American Chemical Society.

Sagarkar, S., Mukherjee, S., Nousiainen, A., Bjorklof, K., Purohit, H. J., Jorgensen, K. S., et al. (2013). Monitoring bioremediation of atrazine in soil microcosms using molecular tools. *Environmental Pollution, 172*, 108–115.

Schiavon, M. (1988). Studies of the leaching of atrazine, of its chlorinated derivatives, and of hydroxyatrazine from soil using 14C ring-labeled compounds under outdoor conditions. *Ecotoxicology and Environmental Safety, 15*, 46–54.

Schröder, P., Harvey, P. J., & Schwitzguébel, J. P. (2002). Prospects for the phytoremediation of organic pollutants in Europe. *Environmental Science and Pollution Research, 9*, 1–3.

Seghers, D., Verthé, K., Reheul, D., Bulcke, R., Siciliano, S. D., Verstraete, W., et al. (2003). Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiology Ecology, 46*, 139–146.

Seiler, A., Brenneisen, P., & Green, D. H. (1992). Benefits and risks of plant protection products possibilities of protecting drinking water: Case atrazine. *Water Supply, 10*, 31–42.

Silva, E,. Fialho, A.M., Sa-Correia I., Burns, R.G., Shaw, L.J. (2004). Combined bioaugmentation and biostimulation to cleanup soil contaminated with high concentrations of atrazine. *Environmental Science & Technology, 38*, 632–637.

Singh, N., Megharaj, M., Kookana, R. S., Naidu, R., & Sethunathan, N. (2004a). Atrazine and simazine degradation in *Pennisetum rhrizosphere*. *Chemosphere, 56*, 257–263.

Singh, P., Suri, C. R., & Cameotra, S. S. (2004b). Isolation of a member of *Acinetobacter* species involved in atrazine degradation. *Biochemical and Biophysical Research Communications, 317*, 697–702.

Smith, D., Alvey, S., & Crowley, D. E. (2005). Cooperative catabolic pathways within an atrazine degrading enrichment culture isolated from soil. *FEMS Microbiology Ecology, 51*, 265–273.

Stamper, D. M., Radosevich, M., Hallberg, K. B., Traina, S. J., & Tuovinen, O. H. (2002). *Ralstonia basilensis* M91-3, a denitrifying soil bacterium capable of using s-triazines as nitrogen sources. *Canadian Journal of Microbiology, 48*(12), 1089–1098.

Stoodley, P., Sauer, K., Davis, D. G., & Costerton, J. W. (2002). Biofilms as acomplex differentiated communities. *Annual Reviews in Microbiology, 56*, 187–209.

Strong, L. C., Rosendahl, C., Johnson, G., Sadowsky, M. J., & Wackett, L. P. (2002). *Arthrobacter auresens* TC1 metabolizes diverse s-triazine ring compounds. *Applied and Environment Microbiology, 68*, 5973–5980.

Struthers, J. K., Jayachandran, K., & Moorman, T. B. (1998). Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. *Applied and Environmental Microbiology, 64*, 3368–3375.

Sui, Y., & Yang, H. (2013). Bioaccumulation and degradation of atrazine in several Chinese ryegrass genotypes. *Environmental Science: Processes and Impacts, 15*, 2338–2344.

Talja, K. M., Kaukonen, S., Kilpi-Koski, J., Malin, I., Kairesalo, T., Romantschuk, M., et al. (2008). Atrazine and terbutryn degradation in deposits from groundwater environment within the boreal region in Lahti, Finland. *Journal of Agriculture and Food Chemistry, 56*, 11962–11968.

Tesar, M., Reichenauer, T. G., & Sessitsch, A. (2002). Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. *Soil Biology and Biochemistry, 34*, 1883–1892.

Tomlin, C. D. S. (2000). *The pesticide manual* (12th ed.). Surrey, UK: The British Crop Protection Council.

Topp, E., Zhu, H., Nour, S. M., Houot, S., Lewis, M., & Cuppels, D. (2000). Characterization of an atrazine degrading *Pseudaminobacter* sp. isolated from Canadian and French agricultural soils. *Applied and Environmental Microbiology, 66*, 2773–2782.

US EPA. (2003). Interim reregistration eligibility decision for atrazine. US: EPA.

Vargha, M., Takats, Z., & Marialigeti, K. (2005). Degradation of atrazine in a laboratory scale model system with Danube river sediment. *Water Research, 39*, 1560–1568.

Vuorimaa, P., Kontro, M., Rapala, J., Gustafsson, J. (2007) *Torjunta-aineiden esiintyminen pohjavedessä* [Occurrence of pesticides in groundwater]. Helsinki: The Finnish Environment 42, Finnish Environment Institute (in Finnish).

Wackett, L. P., Sadowsky, M. J., Martinez, B., & Shapir, N. (2002). Biodegradation of atrazine and related s-triazine compounds: From enzymes to field studies. *Applied Microbiology and Biotechnology 58*, 39–45.

Yassir, A., Rieu, C., & Soulas, G. (1998). Microbial Ndealkylation of atrazine: Effect of exogeneous organic substrates and behaviour of the soil microflora. *Pesticide Science, 54*, 75–82.

Zhang, Y., Jiang, Z., Cao, B., Hu, M., Wang, Z., & Dong, X. (2011). Metabolic ability and gene characteristics of *Arthrobacter* sp. strain DNS10, the sole atrazine-degrading strain in a consortium isolated from black soil. *International Biodeterioration and Biodegradation, 65*, 1140–1144.

Zhou, Z. S., Wang, S. J., & Yang, Z. M. (2008). Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants. *Chemosphere, 70*, 1500–1509.

Index

0-9
1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, 133
1,2- and 1,3-dichlorobenzene (DCB), 201
1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), 191
1,2,4-TCB; 2,5-dichlorophenol (2,5-DCP), 201
1,2-disubtituted epoxides, 5
1,3,4,6-TCDN, 202
18S rRNA sequences, 160
2,2-bis(4-chlorophenyl)ethanol (DDOH), 136
2,2-bis(chlorophenyl)-1-chloroethane (DDMS), 136
2,4,6-Trinitrotoluene – TNT, 62
2,5-DCHQ, 201, 203, 206
2,5-DCP, 33, 202, 206
2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2,5-DDOL), 202
2,5-dichlorohydroquinone(2,5-DCHQ), 39
2,6-dichloro- 1,4-hydroquinone, 37
2-chlorohydroquinone (2-CHQ), 203
2-chlorophenol (2-CP), 203
2-haloaldehydes, 205
3-hydroxychlordane, 141
3-oxoadipate CoA transferase (LinGH), 204
4, 4-diisopropylazobenzene, 95
465-bp *linA* gene, 205
4-chloromethyl, 135
4-isopropyl-hydroxymuconic semialdehyde, 95
6, 7-dihydroxydihydroaldrin, 139
9-hydroxydieldrin, 8, 20, 138

A
A. citreus BI-100, 203
A. giacomelloi, 195
ABC-type transporter, 206
Acetylcholinessterase (AchE), 6
Acetyl-CoA, 202
Achromobacter xylosoxidans strain CS5, 120, 157
Acid phosphatase, 6
Acinetobacter sp, 41
Acromobacter xylosoxidans, 65, 169
Actinobacteria, 195, 206
Actinomycetes sp, 2, 114, 168
Acylhalides, 205
Adenylate energy charge, 114
Adsorption, 16, 36, 87, 88, 116, 175, 200, 215
Aerobacter aerogenus, 172
Agrobacterium sp, 65, 170
Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8, 8a-hexahydro-exo-1,4-endo-5, 8-dimethanonaphthalene), 137
Aldrin dicarboxyric acid, 3, 7, 20
Alkaline hydrolysis, 64, 73, 88
Alkaline phosphatase (ALPase), 6
Allophanate hydrolase, 217
Al-MCM-41, 88
α and γ isomers of HCH, 195
α/β-hydrolase, 205
α-endosulfan, 60, 64, 65, 70, 107, 111, 115, 116, 157, 158
Ammonification, 15
Anabaena azotica, 194
Anaerobic biodegradation pathway, 200
Anaerobic digester sludge, 34
Anaerobic pesticide transformation, 174
Analogues, 12, 132
Aniline, 7, 41, 86, 90, 92, 95, 172
Aniline derivatives, 90, 92
Antiandrogenic activity, 86
Antimicrobials, 131
Antioxidant defence enzymes, 87
Antioxidative defence mechanisms, 75

© Springer International Publishing Switzerland 2017

S.N. Singh (ed.), *Microbe-Induced Degradation of Pesticides*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-45156-5

Aquatic ecosystem, 113, 115
Aromatic ring cleavage, 95, 136
Arthrobacter, 37, 46, 90, 92, 120, 169, 170, 178, 203
Arthrobacter citreus BI-100, 195, 202
Arthrobacter fluorescens, 195, 203
Arthrobacter globiformis D47, 95
Ascomycetes, 93
Aspergillus fumigates, 220
Aspergillus niger, 12, 64, 140, 155, 159
Aspergillus sydowii, 5
Assimilatory sulphate reduction, 118
ATPase, 113
Atrazine $C_8H_{14}ClN_5$, 17, 172, 213–216, 219–221
Atrazine chlorohydralase, 221
Atrazine degradation, 213, 215, 217, 219, 220, 222
Atrazine transformation, 220
*atz*A gene, 217, 220
*atz*abc genes, 219
*atz*abcdef genes, 219
Avena sativa, 197

B
Basidiomycetes, 93
Benzene hexachloride (BHC), 59
β-endosulfan, 60, 64, 65, 69, 72, 107, 111, 115, 116, 119, 120, 151, 155, 160
β-glucorinadase, 5, 19
β-hexachlorocyclohexane, 133
β-isomer, 107, 115, 116, 122, 195
β-ketoadipate pathway, 47, 92, 95
β-ketoadipyl CoA thiolase (LinJ), 204
Bioaccumulation of endosulfan, 115
Bioactivity, 16, 116
Bioaugumentation, 63
Bioavailability, 10, 15, 16, 31, 73, 116, 161, 180, 197, 199, 206
Biocatalysts, 136, 184
Biochemical catalytic capabilities, 180
Bioconcentration factor (BCF), 115
Biodegradation efficiency, 198
Biodegradation of endosulfan, 63, 64, 76, 121, 154, 159
Biodegradation of HCH, 199
Biodegradation of isoproturon, 89, 90, 92, 95, 97
Biodegradation of phenols, 43
Biofertilizers, 184
Biofilm communities, 220
Biofilms, 219
Biological nitrogen fixation, 183
Biological treatment process, 43
Biomarkers, 111
Bio-mineralization, 75, 169
Bioreactors, 63
Bioremediation of endosulfan, 76, 156
Bioremediation technologies, 63, 120
Biosparging, 63
Biostimulation, 63, 197, 221
Biotechnological tools, 194, 207
Biotic metabolism, 66
Biotransformation, 5, 9, 33, 45, 62, 132, 173, 180
Biotransformation products, 39, 154
Bioventing, 63
Biphenyls, 3
Bis(4-chlorophenyl)methane (DDM), 135

C
Candida sp. VITJzN04, 198
Carassius auratus, 113
Carbamate insecticides, 175
Carbon tetrachloride, 107, 108, 145
Carcinogenic effects, 111
Carotenoids, 75
Catabolic enzymes, 196, 217
Catabolic genes, 97
Catabolic plasmids, 169
Catechol, 36, 42, 92, 95, 98, 203
Catechol 1, 2 dioxygenase (C12O), 47
Catechol 2, 3 dioxygenase (C23O), 47
Catechol formation, 98
Cell-cycle kinetics, 111
Cellular membrane, 87
Cetyltrimethyl ammonium bromide (CTAB), 88
Chelatobacter heintzii Cit1, 220
Chemical degradation of endosulfan, 121, 122
Chemical hydrolysis of endosulfan, 118, 122
Chlordane, 20, 106, 115, 132, 140–142, 168
Chlordane isomers, 140
Chlordecone, 133
Chlorella vulgaris, 113
Chlorinated aromatic compounds, 28, 34, 45
Chlorinated cyclodiene pesticides, 1
Chlorinated hydrocarbon insecticides, 175
Chlorinated insecticides, 106
Chlorinated naphthalenes, 133
Chlorinated paraffins, 133
Chlorinated pesticides, 106, 172
Chlorinated phenolic compounds, 28, 31, 34
Chlorinated phenols, 28, 29, 32, 36, 41
Chlorinated solvents, 41, 145
Chlorinated xenobiotics, 28
Chloro-aromatic compounds, 172
Chlorobenzene (CB), 201

Chlorobenzophenone (CBP), 135
Chlorocatechol, 32
Chlorocatechol pathway, 36, 46
Chloroderivatives, 26
Chlorohydrins, 140
Chlorophenolic compounds, 24, 31, 32
Chlorophenols pollution, 28
Chlorotoluron, 90, 91
Chlorpyrifos, 17
Chondrostereum purpureum, 194
Chromosomal aberrations, 111, 152
Cis-muconic acid, 92, 95
Cladosporium oxysporum, 155
Clostridium bifermentans, 3
Clostridium glycolium, 3
Co-factors, 12, 41, 170, 176
Co-metabolic process, 221
Co-metabolic transformation, 41
Co-metabolism, 12, 32, 41, 175, 197
Conformational elasticity, 6
Conjugate pesticides, 172
CPs-degrading microbes, 48
Cunninghamella elegans JS/2, 91, 93
Cyanobacteria, 31, 75, 206
Cyanuric acid, 216, 218, 219
Cyclodiene insecticides, 6
Cyclodiene pesticides, 137
Cypermethrin insecticide, 181
Cytisus striatus, 196, 197
Cytochrome c oxidase, 118
Cytochrome CYP76B1, 177
Cytochrome P450 enzymes, 140
Cytochrome P450 monooxygenase, 7
Cytochrome P450 oxidoreductases, 177

D
DCHQ-1,2-dioxygenase, 37
DDE, 134, 136, 172
DDIPU, 85, 86, 89, 94
DDT (4-, 4′- Dichloro Diphenyl Trichloroethane), 59
DDT-degrading bacteria, 135
DDT mineralization, 135, 136
Dealkylation of amino groups, 218
Dechlorination, 29, 32, 34, 36, 37, 39, 135, 154, 201, 203, 216
Degradation efficiency, 44, 73
Degradation kinetics, 43, 173
Degradation mechanisms, 24, 48
Degradation of endosulfan, 63, 67, 69, 73, 76, 118, 121, 157, 160
Degradation of lindane, 195, 199, 200, 202
Degradation of phenols, 30, 36
Degrading enzymes, 24, 64, 136
Dehalogenases, 178
Dehalogenation, 3, 36, 37, 39, 46, 172, 194
Dehydrochlorinase (LinA), 204
Dehydrogenase enzyme, 75
Deisopropyl atrazine (DIA), 214
Demethylated metabolites, 88
Demethylations of isoproturon, 94
Denitrification, 15
Depolymerization, 221
Desethylatrazine (DEA), 214
Desethyldeisopropylatrazine (DEDIA), 214
Desulfococcus multivorans DSM 2059, 201
Desulfovibrio gigas DSM 1382, 201
Detoxification of endosulfan, 62, 64, 152, 154
Detoxifying enzymes, 7
Dialdehyde, 67, 156
Dichlorobenzhydrol (DBH), 134, 136
Dichlorophenol isomers, 29
Dichlorophenols, 24, 26, 32, 36
Dieldrin (1,2,3,4,10,10-hexachloro-6, 7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1, 4-endo-5,8-dimethanonaphthalene), 137
Dieldrin degradation, 4, 11
Diflubenzuron, 17
Dihydrochlordenedicarboxylic acid, 9, 137, 139
Dihydroxychlordene, 140
Dihydroxydieldrin, 8, 20, 138
Dihydroxydihydrochlordene, 140
Dimethylurea substituted herbicides, 92
Dioxins, 132
Dioxygenase enzymes, 34
Dispersion of isoproturon, 87
Diuron, 90–92
DNA damage, 110, 154
Downstream pathway, 201, 204
Dynamics of isoproturon, 88

E
EC_{50}, 86
Ecotoxicological risks, 45
Electrodylysis, 215
Electron acceptor, 42, 49, 75, 118, 201
Electron donor, 24, 63, 118
Electrophilicity, 205
Endocrine disruptors, 192
Endogenous microorganisms, 169
Endosulfan, 4, 59–64, 66, 68, 70, 73, 75, 76
Endosulfan contamination, 62, 153
Endosulfan degradation, 64, 67, 68, 70, 73, 76
Endosulfan degradation efficiency, 72
Endosulfan dialdehyde, 68
Endosulfan diol, 67, 68, 73, 122, 176
Endosulfan ether, 69, 107, 119, 156, 159, 160

Endosulfan genotoxicity, 111, 153
Endosulfan isomers, 67, 68, 70, 113, 115, 156
Endosulfan lactone, 67, 69, 153, 156, 157, 159
Endosulfan metabolites, 76
Endosulfan monoalcohol, 121
Endosulfan monoaldehyde, 67–69
Endosulfan monooxygenase, 176
Endosulfan residues, 62, 76, 112, 115, 160
Endosulfan sulfate, 66, 70, 75, 76, 113, 151–156, 158, 159, 176
Endosulfandiol endrin, 159
Enrichment technique, 118, 120
Environmental factors, 16, 41, 47, 48, 161
Environmental fate, 29, 66, 73
Environmental pollution, 1, 48
Enzymatic degradation, 76, 168
Enzyme immobilization, 6, 47
Epoxidation reactions, 137
Epoxide hydrolase, 5, 12, 19, 138
Esterases, 170, 178
Extracellular enzymes, 141, 175, 180
Extracellular fluid, 12, 13

F

Facultative anaerobic conditions, 73, 156
Fate of endosulfan, 115, 116
Fate of isoproturon, 86, 87
First order kinetics, 122
Flavonoids, 75
Fluometuron, 90, 92
Free radicals, 5, 75
Fungal metabolism, 139, 158
Fusarium moniliforme, 220
Fusarium poae, 194
Fusarium ventricosum, 65, 155
Fusarium verticillioides, 194, 197

G

GABA (γ-aminobutyric acid), 109
GABA inhibitors, 109
γ-3,4,5,6-TCCH, 201
γ-HCH dechlorination, 201
γ-isomer, 171, 196, 206
γ-pentachlorocyclohexene(γ-PCCH), 202
Genetic engineering technology, 184
Genetic modified microorganism, 168
Genotoxic damage, 152
Glutamic oxaloacetic transaminase, 5, 19
Glutamic pyruvic transaminase, 5, 19
Glutathione, 5, 75
Glutathione S-transferases (GSTs), 5
Glycolipid-type surfactant, 219

H

Haemolymph amino acids, 114
Halidohydrolase (LinB), 204
Haloalkane dehalogenase, 178, 205
Halocatechols, 36
Halogenated compounds, 194, 204
Halomonas campisalis, 42
HCH isomers, 192, 196, 197, 201, 205
HCHs, 193
Hepatic damages, 192
Hepatocarcinogenicity, 86
HepG2 cells, 111
Heptachlor, 6, 140, 141, 143
Heptachlor diol, 141
Heptachlor epoxide, 6, 140, 141, 143
Herbicide-degrading enzymes, 97
Herbicides, 63, 84, 88, 90–92, 131, 168, 170, 175, 178
Hexachlorobenzene, 132, 145, 177
Hexachlorobutadiene, 133
Hexachlorocyclohexane, 132, 168, 171, 178, 191, 195
Hexahydro-1,3,5-trinitro-1,3,5-triazine, 62
Human lymphocytes, 153
Hydrolases, 176, 177
Hydrolytic cleavage, 95
Hydrolytic dehalogenases, 35
Hydrolytic enzymes, 179
Hydrophobic compounds, 200
Hydrophobic partitioning, 42
Hydroxyatrazine, 216, 219
Hydroxyatrazine ethyl amidohydrolase, 217
Hydroxychlordane, 141
Hydroxyisopropyl atrazine, 220
Hydroxylated products, 3, 157
Hydroxylation, 3, 5, 8, 93, 95, 136–138, 142
Hydroxylation reactions, 8, 140
Hydroxymuconicsemialdehyde (HMSA), 203
Hydroxypropyl metabolites, 220
Hyperactivity, 109, 113
Hypoxylon fragiforme, 194

I

Immunosuppressive agent, 114
Indigenous microbes, 97, 155
Ingantiesterogenic activity, 86
Insect-borne diseases, 168
Intermediate metabolites, 2, 19, 201, 202, 204
Ionic pesticide, 175
Isomers, 33, 34, 41, 60, 64, 70, 107–109, 120, 133, 144, 145, 151, 156, 199, 206
Isopropyl alkyl chain, 88

Isoproturon, 84, 86–89, 91–93, 95–98
Isoproturon adsorption, 88
Isoproturon catabolic activity, 89, 92
Isoproturon metabolism, 89, 91, 98
Isoproturon metabolites, 91, 93
Isoproturon mineralization, 89, 92, 96, 97
Isoproturon photocatalytic activity, 88
Isoproturon transformation, 88

K
Ketoaldrin, 2, 5
Klebsiella pneumonia, 65, 155, 156

L
Laccases, 47, 198
Lactic dehydrogenase, 5, 19
Lactone, 75, 112, 157, 158
LC_{50}, 110, 113
LD_{50}, 18, 109, 110, 123
Lemna minor, 86, 89
Leuciscusidus melanotus, 113
Lindane, 17, 106, 160, 168, 172, 183, 191, 192, 194–197, 199, 200, 202, 203, 206
Lindane-degrading bacteria, 195
Lindane remediation, 193
Lin genes, 203
Linuron, 90, 92, 170, 177
Lipid peroxidation, 75
Lipophilicity, 1
Lolium multiflorum, 216

M
Maleylacetate reductase (LinF), 204
Mathematical modeling, 48
MDIPU, 85, 86, 89, 92–95
Membrane properties, 74
Metabolic co-operation, 175
Metabolic pathways, 2, 7, 94, 95, 97, 219
Metabolic transformation, 8, 41
Metabolite biodegradation, 76
Metabolites of endosulfan, 66, 75, 110
Meta-cleavage pathways, 47
Methanogenic bacteria, 206
Methanogenic sludge, 34
Methidathion, 17
Methyl parathion, 17, 120, 170
Methylenedioxyphenyl, 2
Methylopila sp. TES, 90, 91
Microalgae, 31, 89
Microbacterium ITRC1, 195
Microbe-assisted degradation of aldrin and dieldrin, 1
Microbial bioremediation, 168
Microbial consortia, 72, 181, 184, 196, 219
Microbial degradation of endosulfan, 157
Microbial diversity, 15, 181, 182, 221
Microbial kinetics, 43
Microbial toxicity of endosulfan, 74
Microcosm environment, 87
Microencapsulation, 179
Micromonospora sp, 196
Mineralization of isoproturon, 89, 93, 98
Mirex, 106, 132, 143
Mitochondrial dysfunction, 75
Mode of action, 18, 61, 109
Molecular responses, 181
Monochlorinated isomers, 34
Monochlorophenol intermediates, 34
Monochlorophenols, 24, 28, 41
Monodechlorinated, 3
Monohydroxy 6,7-dihydroxydihydroaldrin, 8, 138
Monooxygenase (MO), 5
Monosubstituted epoxides, 6
Mortierella sp. Gr4, 91, 93
Moulting frequency, 113
Mucor racemosus, 4, 5, 138
Mucor thermohyalospora, 64, 159
Mutagenic effects, 110
Mycobacterium chlorophenolicum, 37
Mycobacterium fortuitum, 37
Mycobacterium tuberculosis, 158, 176

N
Natural attenuation, 89, 221
N-dealkylated metabolites, 220
N-dealkylation, 220
N-demethylation, 92–94
N-isopropylammelide, 217, 219
Nitrification, 15, 183, 221
Nitrogen fixation, 169
Non-fluorescent species, 30
Non-persistent insecticides, 168
Non-rhizosphere zone, 173
Non-target species, 114
Non-white rot fungi, 194
Norcardioides, 216
Nutrient cycling, 221
Nutritional factors, 197
Nutritional versatility, 29

O
Ochrobacterium sp. CA1, 219
OCPs, 5, 132, 133
Octahydro - 1,3,5,7 - tetranitro -1,3,5,7 - tetrazocine – HMX, 62
Olfactory learning, 114
Optimal biotic activity, 73

Organ toxicity, 112
Organic xenobiotics, 215
Organochlorinated pesticides (OCPs), 131
Organophosphate compounds, 172
Organophosphate hydrolases, 177
Organophosphate pesticides, 177
Organophosphates, 132, 168
Orgnochlorine insecticides, 168
Ortho-cleavage, 46, 47, 95
Osmoregulation, 114
Oxidation-reduction reaction, 118
Oxidative deamination, 95
Oxidative dechlorination, 142
Oxidative products, 179
Oxidative stress, 75, 85, 152
Oxidative transformations, 179
Oxidoreductase, 9, 170, 176
Oxygenases, 32, 36, 47, 170, 176

P

P. aeruginosa, 30
P. chrysosporium, 45, 135–137, 140, 143, 144
P. florescence, 30
Pandoraea sp, 65, 195, 199
Partition co-efficients ($K_{o/w}$), 26
PCBs, 35, 132
PCP, 32, 34, 36, 39, 44
PCP-4-monooxygenase, 36
*Pdm*AB demethylase genes, 94
Pediculocide, 191
Pedoclimatic factors, 89, 97
Penicillium decumbens, 220
Penicillium miczynskii, 5
Pentachlorocyclohexane, 201
Pentachlorophenol, 24, 38, 46, 48, 62, 133
Peripheral lymphocytes, 152
Periphyton communities, 87
Peroxidases, 47, 179
Peroxidation reactions, 87
Persistent insecticides, 168
Persistent organic pollutants (POPs), 1, 19, 132, 151
Pesticide biodegradation, 199
Pesticide degradation, 9, 154, 160, 169, 172, 174, 175, 179, 184
Pesticide degrading microorganisms, 153, 169, 181
Pesticide mineralization, 174
Pesticide pollution, 168
Pesticide residues, 62, 84, 120, 153, 161, 179, 213
Pesticides, 1, 5, 15–17, 19, 215, 221
pH, 4, 10, 11, 19, 42, 47, 49, 64, 66, 70–74, 84, 88–90, 95, 96, 108, 116, 121, 153, 161, 173–175, 179, 197, 199, 206, 221
Phanerochaete chrysosporium, 46, 66, 68, 135, 155, 159, 220
Phaseolus vulgaris, 14
Phenol degradation kinetics, 43
Phenolic pollutants, 47
Phenylurea herbicides, 90, 91, 95, 177
Phenylurea hydrolase gene *puhA*, 92
Pheromone system, 114
Phlebia brevispora, 3, 136, 137
Phosphate solubilization, 183
Phosphate-solubilizing microorganisms, 16
Phosphotriesterases, 177, 178
Photocatalytic degradation, 88
Photochemical degradation, 29, 109, 122
Photodegradation, 17, 108, 117, 122
Photodegradative reactions, 179
Photodieldrin, 2, 7, 14, 15
photolysis of pesticide residues, 179
Photosensitive pesticides, 17
Photosynthesis ability, 75
Photosynthetic efficiency, 87
Physico-chemical methods, 62
Phyto decomposition, 66
Phytohormones, 183
Phytopathogenic microorganism, 183
Phytoplanktons, 87
Phytoremediation of atrazine, 216
Piloerection, 109, 113
Plant growth hormones, 15, 105
Plasmids, 169
Pleuro tusostreatus, 194
Pleurotus pulmonarius, 220
Polychlorinated biphenyls (PCBs), 132
Polychlorinated dibenzofurans, 3
Polychlorinated dibenzo-p-dioxins, 3
Polychlorinated hydrocarbons, 177
polychlorinated phenols, 36
Polyethylene mulching, 88
POPS, 1, 19, 60, 62, 115, 132, 133
Proton activity, 6
Pseudaminobacter sp, 216
Pseudomonas aeruginosa SFM4, 169
Pseudomonas putida, 30, 36, 41, 46, 170, 172, 177
Pseudomonas sp, 30, 45, 155, 157, 170, 171, 176, 196, 216, 219
PSII, 75, 85
Psychrophilic aquatic bacteria, 16
Psychrotrophic microorganism, 43

Psychrotrophs, 43
Putative active site, 205
Putative metabolic pathways, 215
Pyrene, 200
Pyrethroids, 59, 106, 132, 170, 175

R
Rastonia sp, 216
Reactive oxygen species, 5, 152
Reductive dechlorinase (LinD), 204
Reproductive effects, 112
Residual pesticide, 173
Residues of endosulfan sulphate, 115
Respiratory distress, 109, 113
Reverse osmosis, 215
Rhizoctonia sp, 91, 93
Rhizopus stolonifer, 220
Rhodococcus chlorophenolicus, 45, 46
Rhodotorula sp. VITJzN03, 195, 199
Ring-cleavage dioxygenase (LinE), 204
Root exudates, 196, 197, 216

S
Salicylic acid, 96
Scenedesmus obliquus, 86
Scenedesmus quadricauda, 86
Scenedesmus subspicatus, 86
Scenedesmus vacuolatus, 86
Secondary conjugates, 179, 180
Selective enrichment technique, 119, 120
Sequential anaerobic-aerobic reactors, 43
Sequential UV-biological degradation, 43
Sewage sludge, 34, 96, 137
Sex-linked mutations, 111
Siderophore, 169, 183
Single-cell gel electrophoresis assay, 154
Sodium lauryl sulfate (NaLS), 88
Soil properties, 174, 200, 206
Soil slurries, 199
Solarization, 88
Spatial heterogeneity, 89
Spatial variability, 89, 96
Spent broth, 12
Spent mushroom wastes (SMW), 143
Sphingobium japonicum UT26, 201, 204–206
Sphingomonas chlorophenolicum, 36
Sphingomonas sp. SRS2, 90, 91, 94
Stereoisomers, 18, 151, 191
Stoichiometry of endosulfan, 67
Streptomyces sp. M7, 197–199, 202, 203
Structures of chlorophenols, 24
Sub-lethal effects, 87
Succinate dehydrogenase, 114
Succinyl-CoA, 201
Sulfate metabolites, 113
Superoxide dismutase (SOD), 5

T
TCA cycle, 37
TeCHQ-reductive dehalogenase, 37
Teratogenic effects, 110
Testosterone production, 86
Tetrachlorocyclohexene, 201
Tetramethylenediamine, 136
Thermoactinomyces sp, 3
TiO_2, 88
Tocopherols, 75
Toxicity of endosulfan, 67
Trametes versicolor, 139, 160, 194
Transformation of CPs, 28, 40
Transformation of isoproturon, 86, 88, 93
Tremors, 109, 113
Tricarboxylic acidcycle (TCA), 202
Trichloro-2,5-cyclohexadiene-1-ol (TCCH-1-ol), 203
Trichlorocyclohexadiene (TCCD), 203
Trichlorophenols, 24, 28
Trichoderma harzianum, 17, 64, 155
Trichosporon cutaneum, 31, 36
Tubifex tubifex, 87
Tyrosinases, 47

U
Upstream pathway, 201, 204
US EPA, 115, 214

W
White rot fungi, 3, 8, 31, 45, 68, 175
World Health Organization (WHO), 106, 109, 214

X
Xanthobacter autotrophicus GJ10, 205
Xanthomonas sp, 178, 195, 202

Z
Zygomycetes, 93