

NATIONAL WORKSHOP

**MICROBIOLOGICAL HAZARDS
IN BIOINDUSTRIES -
APPRAISAL, RISK
ASSESSMENT AND
PREVENTION**

March 7-25, 1994

LABORATORY MANUAL



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**Sponsored by
DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA**

L-14 ENVIRONMENTAL IMPACT OF XENOBIOTIC-MICROBE INTERACTIONS

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14.1 Introduction

As the human civilization progressed man started producing more and more synthetic materials including toxic chemicals for his comforts and well being. All these materials after serving his purpose were ultimately dumped into the environment. This continuous and unabated onslaught on the environment has been very severe and ever increasing during the last few decades. However, with the remarkable and unparalleled ability of the nature that is bestowed with the abundant and the most dynamic microbial communities it could, to a great extent, withstand this onslaught. These microorganisms evolved continuously to gain the ability to detoxify and decompose all that was dumped in to it. The remarkable catabolic versatility of microbial communities in soil, water and sewage makes them unquestionably the major decontaminating agents. However, with the advent of all those highly recalcitrant, halogenated and complex chemical compounds which man synthesized and started using indiscriminately for industrial and agricultural purposes and health programmes the scenario started changing. The indigenous microbial communities were not able to cope with the continuous flow of these xenobiotic compounds to its fragile ecosystem, thus resulting in the accumulation of pollutant chemicals in the environment.

These compounds from the environment then started getting in to the food chain eventually reaching the human body. A number of organochlorine pesticides, polychlorinated biphenyls and a number of other industrial chemicals constitute a major bulk of these recalcitrant and toxic xenobiotic compounds. The decomposition of these chemical residues in nature is very slow and, hence, can persist for several years causing serious environmental pollution. The only consoling fact is that nature is so bountiful in its microbial communities and they are continuously evolving genetically to survive in the changing chemical and physical conditions of their habitat. However, it is not advisable to be complacent and leave the task to nature alone. For the last several years scientists all over the world have been involved in finding out methods and in developing technologies for detoxification of chemical industry wastes, sewage etc. before they are disposed off to the environment as well as for decontaminating the already polluted environment. The potential use of degradatory microorganisms for these purposes has been well recognized and a lot of research efforts in this direction have already been made. A number of microorganisms and microbial consortia have been isolated and developed in laboratory for specific pur-

poses. In this article it is intended to give an overview of biodegradation and bioconversion of different groups of pollutant chemicals by microorganisms as well as to highlight some of the undesirable biotransformations taking place in nature.

14.2 The recalcitrance of pollutant chemicals

A chemical compound is called recalcitrant when it is attacked slowly or not at all by microbial systems. Though the recalcitrance cannot be attributed strictly to the structural features of a molecule the following generalization could be made as the cause of recalcitrance :

1. Polymerization, eg. synthetic polymers such as polystyrene, polypropylene, polyvinyl chloride etc.,
2. Introduction of halo-, nitro-, and sulfo-groups or of branched carbon chains into a molecule eg. Pesticides, polychlorinated biphenyls (PCBs), chlorophenols, etc
3. Increasing the number of substitutions of a molecule

Highly recalcitrant compounds include most of the organochlorine pesticides, polychlorinated biphenyls (PCBs), polychlorinated phenols such as pentachlorophenol and a number of other haloaromatics. Pesticides such as hexachlorocyclohexane (BHC), dichlorodiphenyltrichloroethane (DDT), heptachlor, chlordane, aldrin, dieldrin, telodrin etc. can persist in the environment for several years (Khan 1980).

14.3 Pollutant chemicals

Pollutant chemicals are those which cause adverse effects on flora and fauna including man due to their permanent or transitory accumulation in the environment. Their toxic effect on man may be direct or through indirect action on the terrestrial and aquatic ecosystems. Organochlorine insecticides such as BHC (HCH), DDT, aldrin and other industrial chemicals viz. chloroform, carbontetra chloride tri- and tetrachloroethene etc. have been detected in soil, surface and ground water and drinking water, though in levels well below those of acute toxicity. However, due to their persistence and bio-concentration through food chain they reach human body and get accumulated in the adipose tissue in large amounts due to their lipophilic nature. Many of these compounds have been shown to be carcinogenic and teratogenic. Organochlorine pesticides have been shown to cause infertility, pulmonary edema, nervous disorders and various other health problems. In India and many other developing countries high levels of HCH and DDT residues have been reported in human fat. These compounds find their way into human body mainly through food intake. Almost every food article especially dairy products in our country has been shown to be highly contaminated with these toxic chemicals. High levels of these compounds have been detected even in breast milk. Besides these compounds a number of other

compounds are also included in pollutants list. United States Environmental Protection Agency (US-EPA) has prepared a 'list of priority pollutants' after evaluating a lot of data on biodegradability, frequency of occurrence in water, environmental behaviour, toxicity and production capacity of several industrial chemicals (Table 1).

However, the situation in India is slightly different as the amounts and types of industrial and agricultural chemicals produced and used here are different. For example, India is one among the several developing nations where technical grade BHC and DDT are still being used extensively for insect control whereas USA and other advanced countries have banned the use of these chemicals way back in early 1970s. Hence, the environmental pollution with these compounds is much more serious in our country than the developed nations. Pollution with PCBs is more acute in advanced countries than in India.

The entry of pollutants into the environment may be directly from the source in a concentrated form or indirectly in a dispersed manner through the consumer. Fig 1 gives the pathways of pollutant entry to the environment through different routes.

14.4 Microbial degradation of pollutant chemicals

Microbial degradation of chemical compounds in nature may be through aerobic or anaerobic processes. The complete degradation may be effected by a community of several different microbial species or by a single strain, the former being more common. However, some compounds are not getting completely mineralized in nature. They are getting bioconverted in to some other compounds. In many cases the bioconversion results in elimination of the toxicity of the pollutant chemical. However, there are cases where bioconversions lead to the formation of compounds which may be more toxic than the parent compounds. This aspect will be dealt with separately later in this article.

Microbial degradation/transformation of pollutant chemicals may be effected through various metabolic processes. Matsumura (1982) has presented an excellent classification of the types of metabolism of chemical compounds which is as follows:

14.4.1 Enzymatic

14.4.1.1 Incidental metabolism : In this type of metabolism the pollutant chemicals themselves cannot serve as carbon and energy sources to the metabolizing microorganisms.

- i) Metabolism by generally available enzymes.
 - a. Metabolism due to the generally present broad spectrum enzymes (e.g. oxidases, hydroxylases, monooxygenases, dioxygenases, hydrolases etc).

- b. Metabolism due to specific enzymes present in many microbial species.
- ii) Analog-induced metabolism (co-metabolism)
 - c. Metabolism by enzymes utilizing substrates structurally similar to the compound of interest.

All the above three types of incidental biodegradation of pollutant chemicals can, generally, be termed as co-metabolism as this phenomena occurs when the microorganisms naturally grow utilizing some other substrates as carbon and energy sources.

14.4.1.2 Catabolism: In this case pollutant chemicals themselves serve as carbon and energy sources.

- d. The chemicals or a part of the molecule is available as a carbon source.
- e. The chemical is not readily utilized but serves as inducer of specific catabolic enzymes.

14.4.1.3 Detoxification metabolism

- f. Metabolism by resistant microbes.

Thus, we find that the degradation/detoxification of pollutant chemicals in nature takes place through very complex metabolic processes.

Hundreds of bacterial species and several fungal strains have been found to be capable of degrading a bewildering number of synthetic chemicals that are being used and released to the environment. A number of microorganisms capable of degrading different pollutants belonging to a wide array of chemicals groups have been isolated by several workers and thorough investigations on the mechanisms of degradation of specific chemicals have been made (Gibson, 1984; Golovleva et al, 1992, Chaudhry and Chapalamadugu, 1991; Leisinger and Brunner, 1986; Leisinger et al 1981; MacRae, 1989, Matsumura and Krishnamurti, 1982; Lal, 1984; Khan 1980). However, the primary objective of this article is to highlight the fate of the more recalcitrant and toxic compounds.

14.4.2 Degradation through aerobic metabolism

Aerobic metabolism of organic molecule involves molecular oxygen: the transfer of electrons from organic substrates to oxygen generating energy for the cell, and the introduction of oxygen into the substrate which activates it for further metabolism. Majority of the aromatic compounds are catabolised aerobically although there are also few reports of anaerobic degradation of the aromatic nucleus. The fission of the molecular oxygen is catalysed by the enzymes oxidases, monooxygenases and dioxygenases, the

latter two being involved in the oxidation of organic substrates. Monooxygenases act generally on aliphatic compounds whereas dioxygenases are involved in the breakdown of aromatic substances.

14.5 Aliphatic hydrocarbons

The major components of crude oil petroleum products are aliphatic hydrocarbons. Among these n-alkanes especially the long chain alkanes are readily utilized by several strains of Pseudomonas, Arthrobacter, Mycobacterium as sole source of carbon and energy. However, low chain alkanes are more resistant to microbial degradation. Only a few specialized bacterial species can degrade C₁-C₄ alkanes and alkanes in C₅-C₉ range are toxic to many microorganisms. Alicyclic hydrocarbons are more resistant to microbial attack particularly when they are converted into products of commercial interest such as pesticides.

14.6 Halogenated aliphatic compounds

These compounds are extensively used as chemical intermediates and as solvents in a variety of industrial processes, as aerosol propellants, as nematocides and as fumigants. Nearly 150 such compounds are commercially produced and some of them in large quantities. Many of these compounds have been detected in soil, water and air as pollutants. Microbial degradation of a large number of these compounds have been reported (Chaudhry and Chapalamadugu, 1991).

Table 2. gives a list of haloaliphatic compounds degraded aerobically by microorganisms. Strains of Pseudomonas, Moraxella, Xanthobacter etc. have been found to be involved in the degradation.

14.7 Aromatic hydrocarbons

Benzenoid compounds are the most common organic compounds found in nature. These are generally very easily broken down by natural bacteria. However, polycyclic aromatic compounds are more recalcitrant. Condensed polyaromatics having two to four rings are very slowly degraded and those with five or more rings are poor substrates for microbial attack. Introduction of alien substituents to the aromatic nucleus generally makes them more recalcitrant. Substitution with halogens such as chlorine makes aromatic compounds toxic and persistent. PCBs, polychlorophenols, polychlorobenzoates etc. are notorious examples of such pollutant aromatic derivatives. However, in recent years, bacterial strains capable of degrading a number of these compounds including mono-, di-, tri- and tetra-chlorobenzoates, mono-, di-, tri- and penta-chlorophenols and some of the PCBs have been reported. (MacRae, 1989; Chaudhry and Chapalamadugu, 1991).

Most of the alkyl-substituted aromatic compounds such as isomeric xylenes, cresols, xylenols etc. have been shown to be amenable to microbial

degradation. A phenol-degrading *Pseudomonas* sp. Strain CP₄ isolated in our laboratory was found to degrade fairly high levels of all the 3 isomers of cresol and xyleneol.

The critical step in the metabolism of aromatic compounds is the destruction of the resonance structure, that is the fission of the benzenoid ring and this is achieved by dioxygenase-catalyzed reactions in aerobic systems. The benzene rings must carry at least two hydroxyl groups either ortho or para to each other, to be acted upon by ring cleaving enzymes. Based on the substrate that is attacked by the ring-cleaving enzyme dioxygenase the aromatic metabolism can be grouped as catechol pathway, gentisate pathway and protocatechuate pathway. (Fig. 2). In all these pathways, the ring activation by introduction of hydroxyl groups is followed by enzymatic ring cleavage. The ring fission products then undergo further transformations leading to the general metabolic pathways.

14.8 Catechol pathway

Most of the aromatic catabolic pathways converge at catechol. Catechols are formed as intermediates from a vast range of substituted and non-substituted mono- and polyaromatic compounds. The initial reaction of the aromatic compounds is catalyzed by dioxygenases leading to the formation of cis-dihydrodiols which are then converted to catechols by the action of a dihydrodiol dehydrogenase. This reaction involving molecular oxygen occurs mainly in prokaryotes. These dioxygenases are highly labile enzymes and have not been studied in detail. In eukaryotic organisms the initial reaction is catalysed by a monooxygenase forming trans-diol intermediates which derive one hydroxyl group from water.

The catechols are cleaved either by ortho-fission (intradiol i.e. bond between the two hydroxyl groups) or by a meta-fission (extradiol i.e. between one of the hydroxyl groups and a non-hydroxylated carbon) as shown in Fig 2. Most of the dioxygenases involved in the ring fission of catechols have been well characterized.

Generally, substituted catechols are formed from the substituted aromatic compounds eg. chlorobenzenes chlorobenzoates, chlorophenols etc. are converted to the respective chlorocatechols, and toluene, methyl benzoates, cresols etc to the respective methyl catechols.

As a general rule most of the haloaromatics are degraded through the formation of the respective halocatechols the ring fission of which takes place via. ortho-mode. On the contrary most of the non-halogenated aromatics are degraded through meta-pathway. The fission product of ortho-cleavage would be cis-cis-muconic acid or its derivative depending on whether the catechol is substituted or not. The meta-fission product of catechol and substituted catechols would be 2-hydroxymuconic semialdehyde and its substituted derivatives, respectively. The ring-cleavage products of both

ortho- and meta-pathways are further metabolised to intermediates of the tricarboxylic acid cycle. Ortho-pathway is the most productive pathway for the organism as it involves less expenditure of energy.

14.9 Gentisate pathway

In some cases through the action of a monooxygenase aromatic compounds may be converted to gentisic acid. The ring fission in this compound occurs between the hydroxyl and carboxyl group i.e. meta-fission (Fig. 3).

14.10 Protocatechuate pathway

In some cases the chloroaromatic compounds such as 4-chlorobenzoate, 4-chlorophenol and others may get dechlorinated fortuitously during the hydroxylation of the 4th carbon through the action of hydroxylase resulting in the formation of 4-hydroxybenzoate (4-HBA). This 4-HBA will be converted to protocatechuic acid (3,4-dihydroxybenzoic acid) which may be cleaved either through ortho- or through meta-mode (Fig. 4)

14.11 Microbial degradation of haloaromatics

Very large quantities of halogenated aromatic compounds are produced and used as solvents, as chemical feed stock for various chemical synthesis, as lubricants, insulators, pesticides, plasticizers etc. A portion of these chemicals reach the environment and persist due to their recalcitrance. However, microorganisms have been shown to degrade some of them under certain circumstances. Microbial degradation and the biochemical mechanisms of degradation of haloaromatics have been extensively reviewed by several workers (Reineke 1984, Ghosal *et al.*, 1985, Chaudhry and Chapalamadugu, 1991). Table 3. gives a list of chloroaromatic compounds that are degraded by pure microbial cultures.

Though most of the chloroaromatic compounds are recalcitrant and toxic PCBs, pentachlorophenol (PCP) and 2,4,5-T merit a special mention as they have been detected to be major pollutants, particularly in developed countries. PCBs as mixtures of different isomers are used extensively in transformer oils, capacitor dielectrics, heat transformer fluids and many other formulations. Aroclor, Clophen, Phenoclor and Kaneclor are some of the trade names of PCBs. They are highly recalcitrant and persist in the environment for long periods. However, some strains of Acinetobacter, Alcaligenes, Klebsiella and Achromobacter have been shown to be capable of degrading mono-, di- and trichlorobiphenyl. But, PCBs having more than 4 chlorine are resistant to microbial attack. Recently, some bacteria have been shown to degrade some of the PCB isomers co-metabolically (Chaudhry and Chapalamadugu, 1991). Some fungi such as Phanerochacte chrysosporium (lignin-degrader) and Aspergillus niger also have been reported to degrade PCBs.

2,4,5-T is the notorious chemical which was sprayed by Americans under the code Agent orange on the Vietnamese forests and crops which completely

destroyed the vegetation of that country during the war between Vietnam and the U.S.A. It is a very potent defoliating agent particularly of dicotyledons and was being used extensively as herbicides in the Western countries. In our country, however, this compound is not being used. This is also a very persistent compound. In A.M. Chakrabarty's laboratory in California a Pseudomonas cepacia strain AC 1100 was developed by an enrichment technique called "plasmid mediated molecular breeding" which could completely degrade 2,4,5-T. In our laboratory two bacterial strains degrading 2,4,5-T as well as 2,4-D have been isolated, recently. 2,4-D (2,4-Dichlorophenoxy acetic acid) is the herbicide which is commonly used in India. This compound does not cause much pollution problem as it is easily degraded in soil.

Pentachlorophenol (PCP) is a toxic pesticide extensively used for treating timber, leather etc. This compound has been found to be highly carcinogenic. It is highly lethal to fish and other aquatic organisms even at very low concentrations. The use of PCP has been banned in India recently. There are few reports on degradation of PCP by some Pseudomonas spp, Flavobacterium spp., Rhodococcus spp. and Arthrobacter spp. (Chaudhry and Chapalamudugu, 1991).

Extensive work has been done on the degradation of chlorobenzoates. A number Pseudomonas, Arthrobacter, Alcaligenes, Acinetobacter, Corynebacterium, Aspergillus strains have been shown to degrade chlorobenzoates effectively. A P.aeruginosa strain 3mT isolated in our laboratory is capable of degrading 3-chloro- and 4-chlorobenzoates (3-CBA and 4-CBA) upto levels as high as 8g/l and 12g/l, respectively.

Chlorobenzenes are another group of major environmental pollutants as they are extensively used as solvents, fumigants, and precursors in the production of pesticides and dyes. Microbial degradation of these compounds has been rarely found. Recently, some species of Alcaligenes and Pseudomonas have been isolated that can utilize low levels of chlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene as the sole source of carbon and energy.

14.12 Nitroaromatic compounds

These compounds are used in the production of pesticides, dyes, drugs, explosives, and industrial solvents. These compounds are used in large bulks and some of these are toxic. Some of the simpler nitroaromatics such as nitrobenzoic acid, nitroaniline, nitrophenols etc. at low levels are degraded by microorganisms. However, more complex compounds such as 2,4-dinitrotoluene, 2,4,6-trinitrotoluene and others are highly resistant to microbial attack and persist in the environment. These compounds are highly toxic and mutagenic and hence a major threat to life forms.

14.13 Pesticides

A large number of chemical compounds are used as pesticides. On the basis of their chemical nature they may be broadly classified as organophos-

phorus insecticides, carbamates, phenyl-ureas, s-triazines, cyclodienes and organochlorine pesticides and the synthetic pyrethroids. Among these all the non-chlorinated pesticides are generally broken down and detoxified in the environment. A number of books and reviews are available on the microbial degradation of pesticides (Khan 1980, Lal 1984, MacRae 1989, Matsumura and Krishnamurti, 1982, Sethunathan, 1977).

14.13.1 Organophosphorus insecticides

This is a large and the most diverse group of insecticides affecting the nervous system of the insects. Most of these compounds are getting degraded in the environment through microbial action. Parathion (0,0-dimethyl-o-p-nitrophenyl phosphorothioate) is one example of this group of insecticides the microbial degradation of which has been extensively studied.

14.13.2 Carbamates and phenyl-ureas

Isocyanate derived compounds constitute these two groups of pesticides. Both these groups of compounds are generally degraded in the environment (MacRae, 1989). An esterifying enzyme was isolated from a Ps. striata strain which could utilize the carbamate herbicide chlorpropham (iso-propyl-N-(3--chlorophenyl carbamate) showed broad substrate specificity for various carbamate compounds.

14.13.3 s-triazines

The widely used herbicides, s-triazines are utilized by several bacteria as either N or S sources (MacRae 1989).

14.13.4 Organochlorine pesticides

Organochlorine pesticides are highly lipophilic compounds. These insecticides are characterized by three major kinds : DDT analogues, BHC-isomers and cyclodiene compounds. They are broad spectrum insecticides active against a wide variety of insect pests. 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane, commonly known as dichloro diphenyl trichloroethane (DDT) was introduced in 1940 and is considered to be one of the most powerful insecticides and was being used extensively particularly for mosquito control. Though most of the advanced countries have banned the use of DDT way back in 1970s in the developing countries this is still being used. This compound is highly recalcitrant and persists in the environment for several years (Khan, 1980). This compound can be found in almost all compartments of the ecosystem. Hexachlorocyclohexane (HCH) which is commonly known as benzene hexachloride (BHC) exists in a number of isomeric forms and the technical-HCH contains mainly α , β , γ and δ isomers. γ -isomer which is generally referred to as lindane is the only insecticidal component and the technical-HCH contains only 11-18% of this isomer, the rest being the non-insecticidal α , β and δ -isomers which are more persistent and more toxic to non-target organisms. α -isomer may constitute as much as 70% of the technical-HCH. Chlordane, aldrin, dieldrin, heptachlor, toxaphene etc are some other commonly used organochlorine pesticides belonging to the cyclodiene group. These are very powerful insecticides and are highly persistent and

these compounds have been shown to be highly toxic to mammals. Very recently, use of these compounds have been banned in India. Use of DDT for agricultural purpose and spraying of BHC on fruit, vegetable and oil seed crops are also banned in our country. But, our environment has already been polluted very badly with all these chemicals.

DDT is very stable and persistent in well aerated soils. However, in reducing environments and oxygen free atmospheres partial degradation of DDT to various metabolites have been reported. The metabolites detected include DDD, DDE, DDM, etc. Klebsiella aerogenes under anaerobic conditions was shown to convert DDT to DDD. Complete degradation or degradation to non-chlorinated compounds has been very rarely observed. Complete mineralization of DDD and other metabolites of DDT by a Hydrogenomonas sp. has been reported. Some Russian workers have reported the complete degradation of DDT by a sequential anaerobic and aerobic fermentation by P.aeruginosa in the presence of co-substrates such as n-alkanes, glucose, xylose, ribose or glycerol (Golovleva and Skyrabin, 1981). Co-metabolic conversions and degradation of DDT and its catabolic intermediates by a few bacterial and fungal (Aspergillus conicus, A.niger and Penicillium brefedatum) isolates to varying degrees have also been reported (Matsumura and Krishnamurti, 1982; MacRae, 1989). A lignin-degrading white-rot fungus Phanerochaete chrysosporium has been shown to degrade completely a number of persistent chemicals such as DDT, lindane, 2,3,7,8-tetrachlor-dibenzo-p-dioxin, 2,4,5,2'4'5'-hexachlorobiphenyl and others (Bumpus et al, 1985). However, all these processes are very slow.

Reports on biodegradation of HCH are rather scanty. A majority of the available reports mainly deal with anaerobic degradation of HCH in soil under flooded conditions (MacRae, 1989; Sethunathan et al, 1991). Clostridium spp have been implicated to be involved in the anaerobic biodegradation of r-HCH.

A few reports are available on aerobic biodegradation of HCH-isomers. By repeated additions of HCH to a rice field under flooded and non-flooded conditions Sethunathan's group was able to get the soil microorganisms acclimatized which could readily degrade r-HCH under aerobic conditions (Sethunathan et al., 1991). They also isolated a Pseudomonas sp. from the rhizosphere of HCH-treated sugar cane plants which could degrade all the four major isomers of HCH viz., α , β , γ and δ , aerobically. Very recently they have reported isolation of bacterial strain, Sphingomonas paucimobilis from HCH-acclimatized soil which also degraded all the 4 isomers of HCH. We have developed different microbial consortia which can degrade fairly high levels of different HCH-isomers under aerated conditions (Chandrasekharaiah, 1993). One of these consortia can degrade upto 50 ppm of α - and r-HCH and upto 10 ppm of β and δ -isomers. Japanese workers have reported isolation of Ps. paucimobilis strain SS86 capable of utilizing r-HCH.

Reports on complete biodegradation of chlorinated cyclodiene pesticides does not seem to be available. However, bioconversion of aldrin to

dieldrin, heptachlor to heptachlor epoxide etc in soil have been reported. The fungal and bacterial strains that are involved in these biotransformation processes have been shown to be A. niger, A. flavus, P. notatum, P. chrysogenum, species of Fusarium, Trichoderma, Nocardia, Streptomyces, Micromonospora, Bacillus and Pseudomonas. A faster degradation of endrin in flooded soils than in non-flooded soils also has been reported.

Endosulfan is another highly chlorinated pesticide which is used in our country. This compound is also highly persistent. There are only very scanty information on the complete mineralisation of this compound. However, microbial transformation of endosulfan to endosulfan diol has been reported by several workers. Microalgae viz., Chlorella, Scenedesmus fungi, viz., Penicillium spp., Aspergillus spp., Botrytis cinerea, Papularia sp. and bacteria viz., Nocardia, Corynebacterium, Bacillus, Pseudomonas etc have been implicated to be responsible for this bioconversion.

14.14 Surface active agents

Surface-active agents commonly called surfactants are synthetic chemicals used for cleansing purposes. These are one of the major components of the modern detergents. In India too these detergents have almost replaced the traditional soaps. The first generation detergents containing the surfactants, tetrapropylene-derived alkyl benzene sulfonates were causing pollution problem in the Western world. Now the new generation detergents (soft-detergents) containing more biodegradable linear alkylbenzene sulfonates (LAS) have replaced the 'hard-detergents'. However, the detergents containing alkyl ethoxylates, alkyl phenol ethoxylates with more than 20 ethoxylate units have been found to cause environmental problems as they are slowly and partially degraded leaving behind the toxic alkyl phenols.

14.15 Synthetic dyes

More than 40,000 dyes and pigments having about 7000 different chemical structures have been synthesised of which about 3500 are in practical use especially in textile industry. Most of these dyes are not very harmful environmentally except benzidine dyes and triphenylmethane type. Studies on microbial degradation of dyes have been conducted only with some of the azo dyes. Mixed bacterial communities were shown to degrade these dyes.

14.16 The negative roles of microorganisms

So far we have been looking into the positive roles of microorganisms in eliminating or abating the pollution problems by degrading/transforming pollutant chemicals. However, there are a number of negative roles also that microorganisms play in nature. The biodeterioration of various materials by microbial growth and degradation is one main example of such a role. Wood, textile, paper, paint, leather, and a wide variety of materials of use may become the target of microbial spoilage under congenial conditions for microbial growth. A wide array of bacteria, fungi, and microalgae have been found to be involved in spoilage of different materials.

Besides these there are some other negative roles that are played by microorganisms. Some of the biotransformations that take place in the environment may turn to be deleterious. Biotransformations of a number of pollutant chemicals have been shown to be resulting in the formation of more toxic and more persistent compounds. For example, chlorocyclohexene insecticide aldrin is generally converted to dieldrin through microbial mediation. Dieldrin is more toxic and recalcitrant than aldrin. DDT is usually biotransformed in soil to DDE which is more toxic than the parent compound. Heptachlor, another organochlorine pesticide is generally bioconverted to heptachlor epoxide which is more reactive and toxic. Through the mediation of microbial enzymes trichloroethylene will be converted to organic intermediates such as vinyl chloride which is highly carcinogenic and toxic. Nitroaromatic compounds and azo dyes may get reduced by anaerobic microbes to aromatic amines which are highly persistent and carcinogenic. Partial degradation of surfactant chemicals such as nonylphenol-polyethoxylate usually result in the accumulation of the toxic alkyl phenol.

Generally chloroaromatic compounds such as chlorobenzoates, chlorophenols, etc get degraded through the formation of chlorocatechols as intermediate compounds. Other haloaromatics also form halocatechols as intermediates. It has been shown that a number of bacteria are capable of converting chloroaromatics to chlorocatechols. But, many of them get inhibited once halocatechols are formed. Chlorocatechols strongly and irreversibly inhibit the catechol-2,3-dioxygenases, the enzymes that cleave the aromatic ring through meta-mode. They also affect the ortho-cleaving enzymes catechol 1,2-dioxygenases. For the degradation of chlorocatechols a modified ortho-pathway has to be present in the organisms which is rare. Hence, chlorocatechols accumulate in the environment. They get autooxidized and polymerized spontaneously forming a dark brown coloured complex. The dark brown colouration of the sewage that is generally observed is due to this phenomenon. As halocatechols inhibit the meta-cleaving enzymes the degradation of non-chlorinated aromatic compounds such as benzoates, phenolic compounds, the methyl derivatives such as cresols, etc also will be affected resulting in the accumulation of most of the polluting chemicals.

14.17 Conclusion

The new generation synthetic chemicals are highly complex, derivatized and polymerized and hence, are more recalcitrant and persistent in the environment. Though native microflora of soil, water bodies and sewage are highly biochemically versatile, many a times by failure to degrade some of the toxic and recalcitrant xenobiotics such as organochlorine pesticides, PCBs and other polyhaloorganic compounds. Due to continuous and prolonged exposure to these compounds, native microbial communities have attained ability to degrade many of the xenobiotic compounds, some rapidly and some very slowly. Some microbial transformations also lead to the formation of more toxic and more recalcitrant compounds. There is a need to develop treatment technologies for decontamination and elimination of pollutants from industrial effluents, soil and sewage by deploying superior genetically engineered microorganisms.

Table-1: Organic chemicals on the EPA list of priority pollutants

Chemical class (No. of compounds)	Important representative and their frequency of occurrence
Pesticides and metabolites (17)	B-hexachlorocyclohexane (0.8%)
Halogenated aliphatics (31)	Dichloromethane (36%) Trichloromethane (37%)
Aromatics (8)	Benzene (26%); Toluene (28%)
Nitroaromatics (7)	2-Nitrophenol (2%) Nitrobenzene (2%)
Chloroaromatics (16)	Pentachlorophenol (7%) Dichlorobenzene (6%)
Polychlorinated biphenyls (7)	Aroclor 1254 (0.6%) Aroclor 1242 (0.8%)
Phthalate esters (6)	Bis-(2-ethyl hexyl) phthalate (42%), Di-n-butylthalate (19%)
Polycyclic aromatic hydrocarbons	Phenanthrene/anthracene (11%) Pyrene (8%)
Nitrosamines and miscellaneous compounds (6)	Diphenyl nitrosamine (1%)

Frequency of occurrence in 2600 waste water samples from different industrial categories. (Data from Leisinger & Brunner, 1986).

Table-2: Haloaliphatic compounds degraded aerobically by microorganisms (Leisinger and Brunner 1986)

Halogenated hydrocarbons	2-Haloalkanoic acids
Dichloromethane	Monochloroacetate
1,2-Dichloroethane	Dichloroacetate
Chloroethane	Trichloroacetate
1-Chloropropane	L-2-Chloropropionate
1,3-Dichloropropane	2,2-Dichloropropionate
1-Chlorobutane	DL-2-Bromobutyrate
1,9-Dichloronane	DL-2-Bromovalerate.
1-Chloroheptane	

Table-3: Chloroaromatic compounds degraded by bacterial cultures

2-chlorobenzoate,	2-Chlorophenol
3-Chlorobenzoate,	3-Chlorophenol
4-Chlorobenzoate,	4-Chlorophenol
2,4-Dichlorobenzoate,	2,4-Dichlorophenol
3,4-Dichlorobenzoate	2,4,6-Trichlorophenol
3,5-Dichlorobenzoate.	
P-Chlorophenoxy acetate,	Pentachlorophenol
2,4-Dichlorophenoxy acetate (2,4-D)	Chlorobenzene
2,4,5-Trichlorophenoxy acetate (2,4,5-T)	Monochlorobiphenyl
2-Methyl-4-Chlorophenoxy acetate (MCPA)	Dichlorobiphenyl
2-Chlorotoluene	Trichlorobiphenyl.
3-Chlorotoluene	
4-Chlorotoluene	
2,4-Dichlorotoluene	
3,4-Dichlorotoluene	

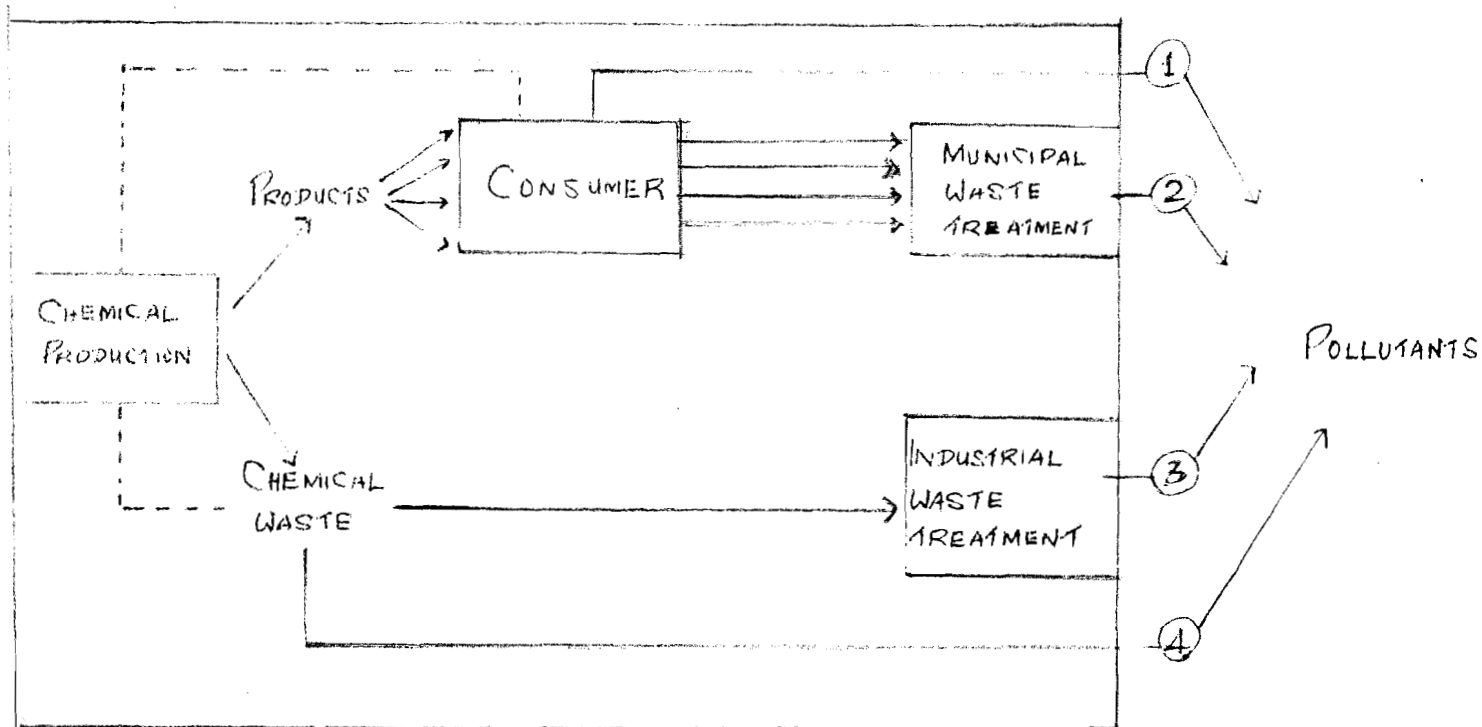
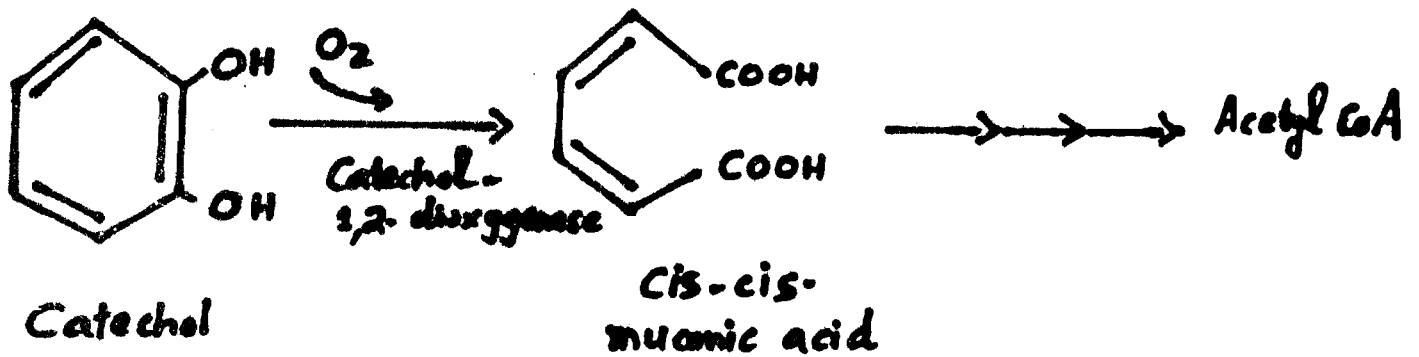


Fig 1. Release of organic chemicals to the environment.

1. Chemicals whose use leads to their entry into the environment, e.g., aerosol propellants, pesticides, fertilizers.
2. Chemicals entering the environment in the effluents of municipal sewage treatment systems, e.g., hard detergents, solvents.
3. Chemicals resistant to biological degradation in industrial waste treatment systems, e.g., chlorobenzenes, amino naphthol, sulfonic acids.
4. Direct discharge, losses, spills and accidents leading to the entry of chemicals from production sites into the environment.

(Fig. from Lelsinger and Brunner 1986).

A. ORTHO-CLEAVAGE



B. META-CLEAVAGE

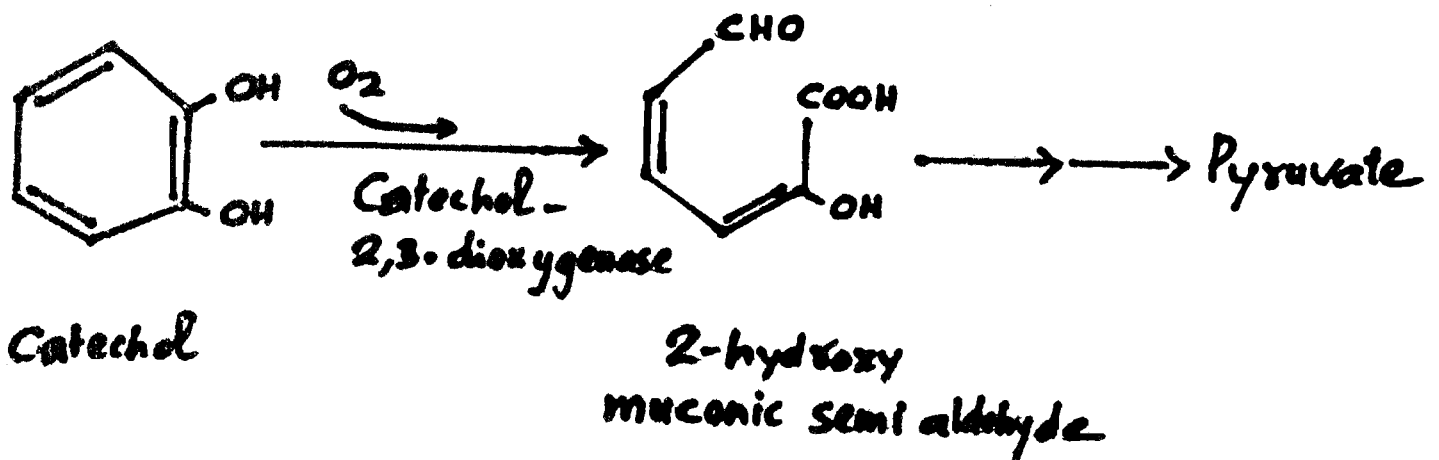


Fig. 2. RING CLEAVAGE OF AROMATIC COMPOUNDS.

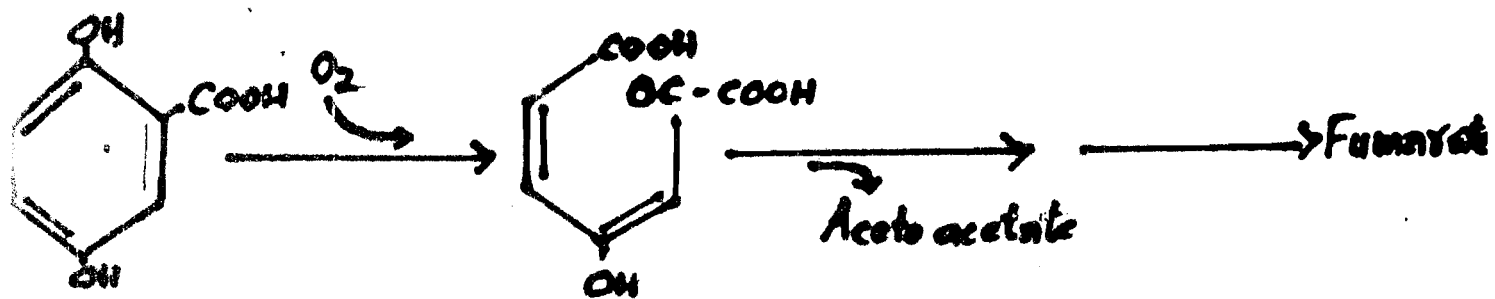


Fig. 3. RING FISSION OF GENTISIC ACID AND FURTHER DEGRADATION.

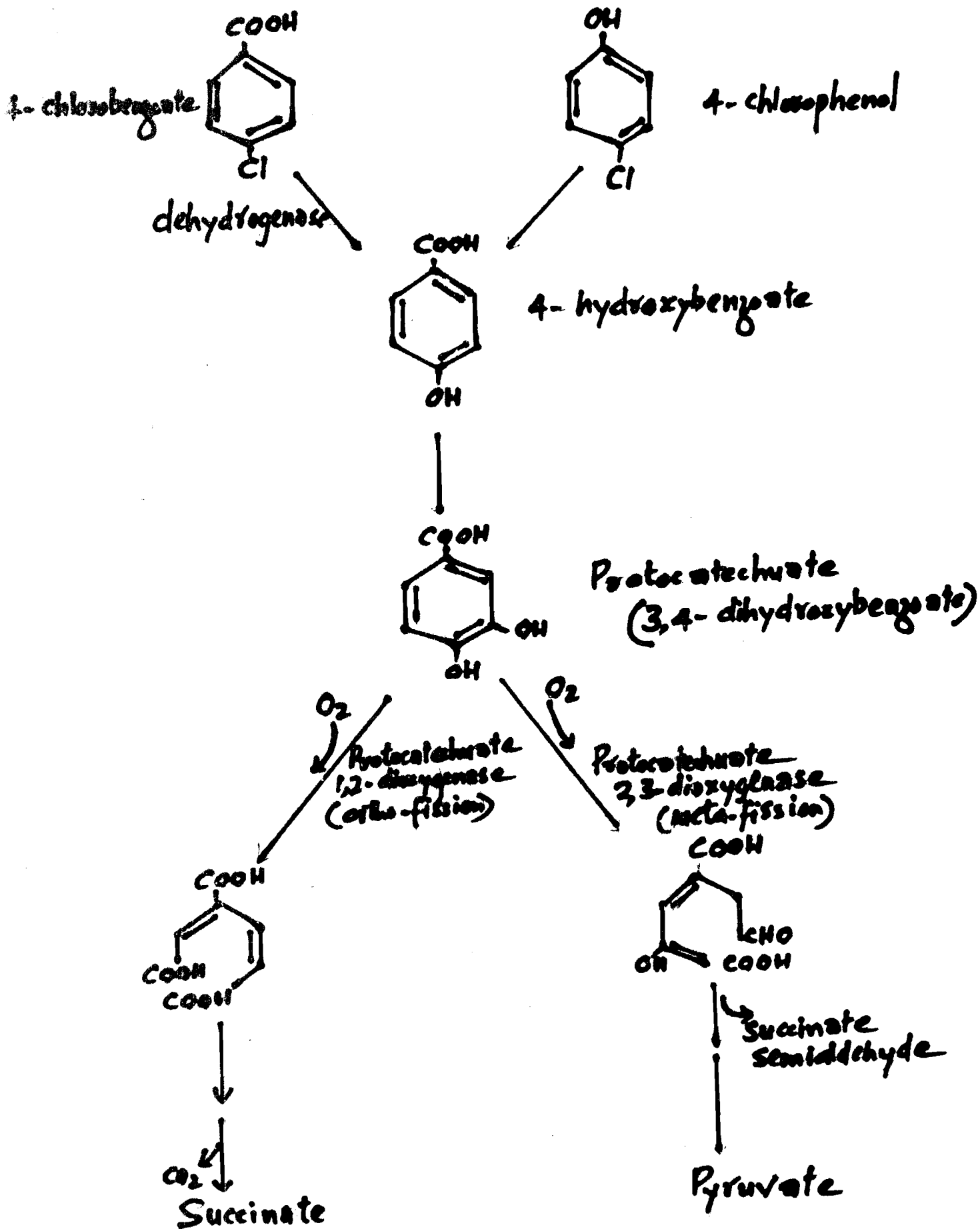


Fig. 4. PROTocatechuate PATHWAY OF DEGRADATION OF CHLOROAROMATICS.

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