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Review

Biodegradation of phenol

C. Indu Nair, K. Jayachandran* and Shankar Shashidhar

School of Biosciences, M.G.University, Kottayam, Kerala, India 686560.

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The use of microbial catalysts in the biodegradation of organic compounds has advanced significantly during the past three decades. It has been found that large numbers of microbes co-exist in almost all natural environments, particularly in soils. Many natural and synthetic organic chemicals are readily biodegradable in natural environment. Biodegradation of materials involve initial proximity, allowing adsorption or physical access to the substrate, secretion of extra cellular enzymes to degrade the substrates or uptake via transport systems followed by intracellular metabolism. The efficiency of biodegradation of organic compounds is influenced by the type of the organic pollutant, the nature of the organism, the enzyme involved, the mechanism of degradation and the nature of the influencing factors. Phenolic compounds are hazardous pollutants that are toxic at relatively low concentration. Accumulation of phenol creates toxicity both for flora and fauna. Since phenol is toxic and cause pollution, it must be removed from the environment.

Key words: Biodegradation, organic compounds, pollution.

INTRODUCTION

Organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health. Many of these are resistant to degradation. As they persist in the environment, they are capable of long range transportation, bioaccumulation in human and animal tissue and biomagnification in food chain.

Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds (Atlas and Bartha, 1998). The term has been proposed for describing the ultimate degradation and recycling of an organic molecule to its mineral constituents. According to Alexander (1965) no natural organic compound is totally resistant to biodegradation provided that the environmental conditions are favourable. This is known as the principle of microbial infallibility.

Microbiologists have hardly dipped below the surface of the natural pool of microbial diversity. When new organisms have been isolated with biodegradation efficiency, their biochemical versatility has been found to be immense. Attempts to determine microbial diversity in natural environments like soil are limited by the inability of the microbiologists to culture specific microbes present in a particular environmental sample. However, the isolation of those microbes will often require a targeted intelligent approach to screen the biosphere for its presence (Wackette and Hershberger, 2001).

The massive mobilization of compounds in natural resources or the introduction of xenobiotics into the biosphere leads to unidirectional fluxes, which result in the persistence of a number of chemicals in the biosphere and thus constitute a source of contamination. Phenol and its higher homology are aromatic molecules containing hydroxyl group attached to the benzene ring structure. The origin of phenol in the environment is both industrial and natural. Phenol pollution is associated with pulp mills, coal mines, refineries, wood preservation plants and various chemical industries as well as their wastewaters (Paula and Young, 1998). Natural sources of phenol include forest fire, natural run off from urban area where asphalt is used as the binding material and natural decay of lignocellulosic material. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Ghadhi and Sangodkar, 1995). Phenols are toxic to human beings and affect several biochemical functions (Nuhoglu and Yalcin, 2005). Phenol is also a priority pollutant and is included in the list of EPA (1979).

*Corresponding author. E-mail: jayan_chk@rediffmail.com.

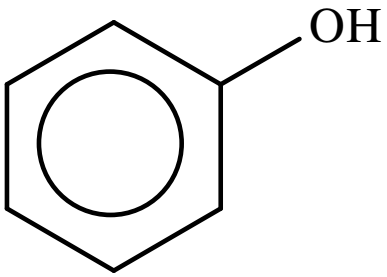

Figure 1. Chemical structure of phenol.

CHEMISTRY OF PHENOL

Synonyms

Carbolic acid, Hydroxybenzene, phenic monohydroxybenzene, phenic acid, phenylic acid, phenyl hydroxide, oxybenzene, benzenol, monophenol, phenyl hydrate, phenylic alcohol, Baker's P and S, phenol alcohol.

Chemical formula (C_6H_6O)

Phenols contain an OH group attached directly to an aromatic ring (Figure 1).

Properties

They may be colourless solids or thick liquids, often contains a pink tint owing to the presence of oxidation products. Phenol is a hygroscopic, crystalline solid with distinctive odour and is acidic. Molecular weight of phenol is 94.11, the density is 1.072 and the boiling point is 181.9°C.

TOXICITY OF PHENOL

Acute exposure of phenol causes central nervous system disorders. It leads to collapse and coma. Muscular convulsions are also noted. A reduction in body temperature is resulted and this is known as hypothermia. Mucus membrane is highly sensitive to the action of phenol. Muscle weakness and tremors are also observed. Acute exposure of phenol can result in myocardial depression. Phenol causes a burning effect on skin. Whitening and erosion of the skin may also result due to phenol exposure. Phenol has an anaesthetic effect and causes gangrene. Renal damage and salivation may be induced by continuous exposure to phenol.

Exposure to phenol may result in irritation of the eye, conjunctional swelling, corneal whitening and finally blindness. Other effects include frothing from nose and mouth followed by headache. Phenol can cause hepatic damage also. Chronic exposure may result in anorexia, dermal rash, dysphasia, gastrointestinal disturbance, vomiting, weakness, weightlessness, muscle pain, hepatic tenderness and nervous disorder. It is also suspected that exposure to phenol may cause paralysis, cancer and genetofibre striation. Phenol and its derivatives are toxic and classified as hazardous materials (Zumriye and Gultac, 1999). These phenolic compounds possess various degrees of toxicity and their fate in the environment is therefore important (Bollag et al., 1988). In recent years, a great deal of research work has been directed toward the development processes in which enzymes are used to remove phenolic contaminants (Ghioureliotis and Nicell, 1999). Phenol is an antiseptic agent and is used in surgery, which indicates that they are also toxic to many microorganisms (EPA, 1979).

MICROORGANISMS IN PHENOL BIODEGRADATION

Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria, fungi and actinomycetes (Table 1). Bacterial species include *Bacillus sp*, *Pseudomonas sp*, *Acinetobacter sp*, *Achromobacter sp* etc. *Fusarium sp*, *Phanerocheate chrysosporium*, C*orious versicolor*, *Ralstonia sp*, *Streptomyces sp* etc are also proved to be efficient fungal groups in phenol biodegradation. However, these microorganisms suffer from substrate inhibition at higher concentration of phenol, by which the growth is inhibited (Prieto et al., 2002).

Many studies on biodegradation of phenol come from bacteria. The genus *Pseudomonas* is widely applied for the degradation of phenolic compounds. These bacteria are known for their immense ability to grow on various organic compounds. Phenol biodegradation studies with the bacterial species have resulted in bringing out the possible mechanism and also the enzyme involved in the process. The efficiency of the phenol degradation could be further enhanced by the process of cell immobilization (Annadurai et al., 2000a, b). Phenol and other phenolic compounds are common constituents of many industrial effluents. Once a suitable micro organism based process is developed for the effective degradation of phenol these phenolic effluents can be safely treated and disposed (Borghei and Hosseini, 2004). *Candida tropicalis* RETL-Crl from the effluent of the Exxon Mobile Oil Refinery waste water treatment was investigated for phenol degradation using batch and fed batch fermentation under aerobic condition (Mohd Tuah, 2006). Microbiological degradation of phenol and some of its alkyl derivatives (p-cresol, 4-n-propyl phenol, 4-i -propyl phenol, 4-n-butyl phenol, 4-sec-butyl phenol, 4-t-butyl phenol and 4-t-octyl phenol) were examined under both aerobic and anaecrobic conditions in seven Japanese paddy soil samples (Atsushi et al., 2006). The rate of biodegradation of phenol by *Klebsiella oxytoca* strain was studied. It was found that *K. oxytoca* degraded phenol at elevated concentration where 75% of initial phenol con-

Table 1. Microorganisms in the biodegradation of phenolic compounds.

S/N	Type of phenol	Microorganisms	Reference
1	Phenol	*Bacillus stearothermophilus*	Gurujeyalakshmi and Oriel (1988)
2	Phenol	*Pseudomonas putida*	Allsop et al. (1993)
3	Phenol	*Agaricus bisporus*	Burton et al. (1993)
4	Pentachlorophenol	*Lentinus bisporous*	Okeke et al. (1993)
5	Phenol	Aerobic consortium	Ambujam and Manilal(1995)
6	Phenol	*Acinetobacter johnsonii*	Hoyle et al. (1995)
7	2- cholrophenol	*Pseudomanas putida*	Overmeyar and Rehm (1995)
8	Phenol	*Pseudomonas sp*	Bodzek et al. (1996)
9	Phenol	*Pseudomonas sp*	Gotz and Reuss(1997)
10	Penta, chlorophenol	*Lentinula edodes*	Okeke et al. (1997)
11	Phenol	*Ochromonas danica*	Semple and Cain(1997)
12	Phenol	*Phormidium valderianum*	Shashirekha et al. (1997)
13	Phenol	*Bacillus* sp	Ali et al. (1998)
14	Phenol	*Rhizoctonia praticola*	Bollag et al. (1988)
15	Phenol	*Trametes trogii*	Garzillo et al. (1998)
16	Phenol	*Pseudomonas putida*	Loh and Wang (1998)
17	Phenol	*Pseudomonas flurorescens*	Torres et al. (1998)
19	Phenol	*Pseudomonas putida*	Mordocco et al. (1999)
20	Phenol	*Coriolus versicolor*	Kadhim et al. (1999)
21	Phenol	*Ralstonia eutropha*	Leonard et al. (1999 a,b)
22	Phenol	*Coprinus cinereus*	Schneider et al. (1999)
23	Phenol	*Pseudomonas putida*	Wang and Loh (1999)
24	Phenol	*Pseudomonas putida*	Zumriye and Gultac (1999)
25	Phenol	*Pseudomonas pictorium*	Annadurai et al. (2000)
26	Phenol, Nitrophenol	*Nocardioides*	Cho et al. (2000)
27	Phenol	*Phanerocheate chrysosporium*	Garcia et al. (2000)
28	Phenol	*Pleurotus ostreatus*	Hublik and Schinner (2000)
29	Phenol	*Pseudomonas putida*	Loh and Tar (2000)
30	Phenol	*Acinetobacter calcoaceticus*	Nakamura and Sawada (2000)
31	Phenol	*Chalara paradoxa*	Robles et al. (2000)
32	Phenol	*Streptomyces setonii*	An et al. (2001)
33	Phenol	*Alcaligenes sp*	Baek et al. (2001)
34	Phenol	*Pseudomonas sp*	Gonzalez et al. (2001)
35	Phenol	*Pseudomonas putida*	Loh and Jun (2001)
36	Phenol	*Pseudomonas putida*	Petruschka et al. (2001)
37	Bisphenol A	*Coprinus cinereus*	Sakurai et al. (2001)
38	Phenol,	*Acinetobacter sp*	Hao et al. (2002)
39	Phenol	*Rhodococcus erythropolis*	Prieto et al. (2002)
40	Phenol	*Trichosporon cutaneum*	Godjevargova et al. (2003)
41	Phenol	*Termitomyces albuminosus*	Johjima et al. (2003)
42	2, 4 dichloro phenol	Mixed culture	Quan et al. (2003)
43	Chloro phenol	*Pseudomonas putida*	Farighian (2003)
44	Chloro phenol	*Achromobacter sp*	Xiangchun et al. (2003)
45	Phenol	Mixed Fungi	Atagana et al. (2004)
46	Phenol	*Pseudomonas putida*	Hamed et al. (2004)
47	Phenol	*Alcaligenes sp*	Nair and Shashidhar (2004)
48	Phenol	*Fusarium sp*	Santos and Linardi (2004)
49	Pentachlorophenol	*Sphingomonas chlorophenolica*	Bielefeldt and Cort (2005)
50	Dichlorophenol	*Pseudomonas putida*	Kargi and Eker (2005)
51	Phenol	*Pseudomonas sp*	Prpich and Douglis (2005)
52	Phenol	*Bacillus brevis*	Arutchelvan et al. (2006)
53	4. Nonyl phenol	*Clavariopsis aquatica*	Moeder et al. (2006)

Table 2. Enzymes involved in the biodegradation of phenolic compounds.

S/N	Type of Phenol	Enzyme	Reference
1	Phenol	Phenol hydroxylase	Gurujeyalakshmi and Oriel (1988)
2	Phenol	Polyphenol Oxidase	Burton et al. (1993)
3	Phenol	Polyphenol Oxidase	Cano et al. (1997)
4	Phenol	Phenol Oxidase	Okeke et al. (1997)
5	Phenol	Polyphenol oxidase	Shashirekha et al. (1997)
6	Phenol	Catechol 2,3 dioxygenase	Ali et al. (1998)
7	Phenol	Laccase	Bollag et al. (1998)
8	Phenol	Polyphenol oxidase	Garzillo et al. (1998)
9	Phenol	Peroxidase	Ghioureliotis and Icell (1998)
10	Phenol	Horse radish peroxidase	Wu et al. (1998)
11	Phenol	Horse radish peroxidase	Zahida et al. (1998)
12	Phenol	Polyphenol oxidase	Edwards et al. (1999)
13	Phenol	Laccase	Kadhim et al. (1998)
14	Phenol	Laccase	Schneider et al. (1999)
15	Methoxyphenol	Laccase	Setti et al. (1999)
16	Phenol	Laccase	Hublik and Schinner (2000)
17	Phenol	Laccase	Robles et al. (2000)
18	Phenol	Catechol 1,2oxygenase	An et al. (2001)
19	Phenol	Polyphenol oxidase	Luke and Burton (2001)
20	Bis phenol	Peroxidase	Sakurai et al. (2001)
21	Phenol	Polyphenol oxidase	Steffens (2002)
22	Phenol	Phenol oxidase	Johjima et al. (2003)
23	Phenol	Tyrosinase	Xiangchun (2003)
24	Lignophenols	Peroxidase	Xia et al. (2003)

centration at 100 ppm was degraded within 72 h (Shawabkeh et al., 2007). Phenol was degraded by *Actinobacillus species (*Khleifat and Khaled, 2007). They found that pH 7, the incubation temperature of 35 to 37°C, and the agitation rate of 150 rpm were the optimal conditions for achieving the higher percentage of phenol degradation. Succinic acid and glycine as respective carbon and nitrogen source were found to be the most efficient co-substrates for the removal of phenol. Immobilized *Alcaligenes sp* d_2 was successfully used for the effective treatment of phenolic paper factory effluent (Nair and Shashidhar, 2007).

MECHANISM OF PHENOL BIODEGRADATION

Generally aromatic compounds are broken down by natural bacteria. However, polycyclic aromatic compounds are more recalcitrant. Derivatisation of aromatic nuclei with various substituents particularly with halogens makes them more recalcitrant. There are reports on many microorganisms capable of degrading phenol through the action of variety of enzymes. These enzymes may include oxygenases hydroxylases, peroxidases, tyrosinases and oxidases (Table 2).

Oxygenases include monoxygenases and dioxygenases. The critical step in the metabolism of aromatic compounds is the destruction of the resonance structure by hydroxylation and fission of the benzoid ring which is achieved by dioxygenase-catalysed reactions in the aerobic systems. 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 proto catechaute pathway. In all these pathways, the ring activation by the introduction of hydroxyl groups is followed by the enzymatic ring cleavage. The ring fission products, then undergoes transformations leading to the general metabolic pathways of the organisms. 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 poly aromatic compounds. Aerobically, phenol also is first converted to catechol, and subsequently, the catechol is degraded via ortho or meta fission to intermediates of central metabolism. The initial ring fission is catalysed by an ortho cleaving enzyme, catechol 1, 2 dioxygenase or by a meta cleaving enzyme catechol 2,3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydro cis muconic semi aldehyde for the latter (Gurujeyalakshmi and Oriel, 1988).

Streptomyces setonii (ATCC 39116) degraded aromatic

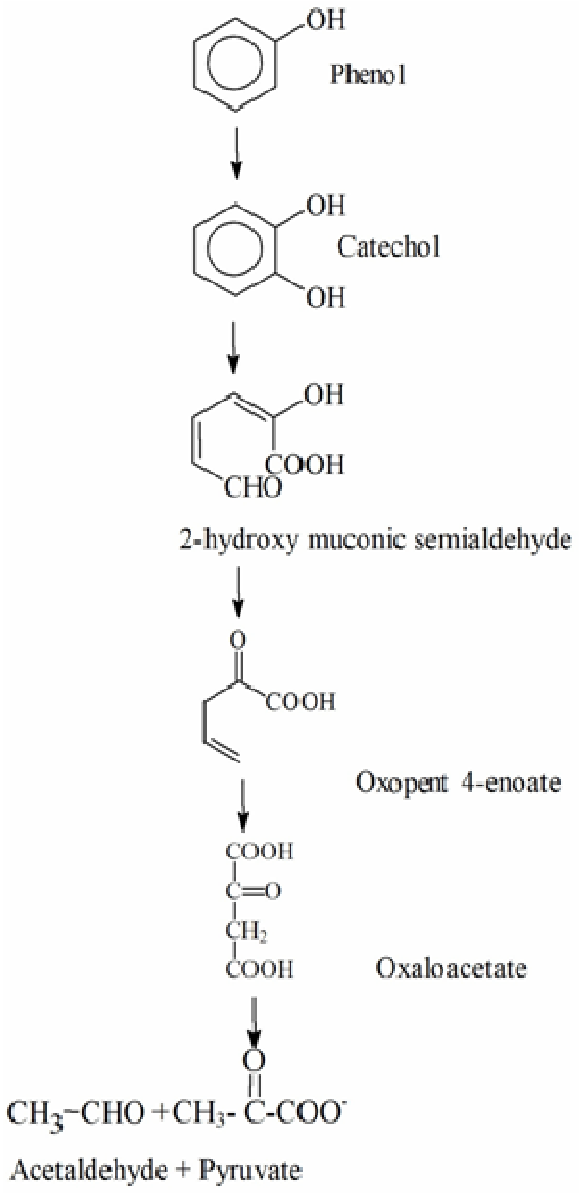

Figure 2. Meta pathway of phenol degradation.

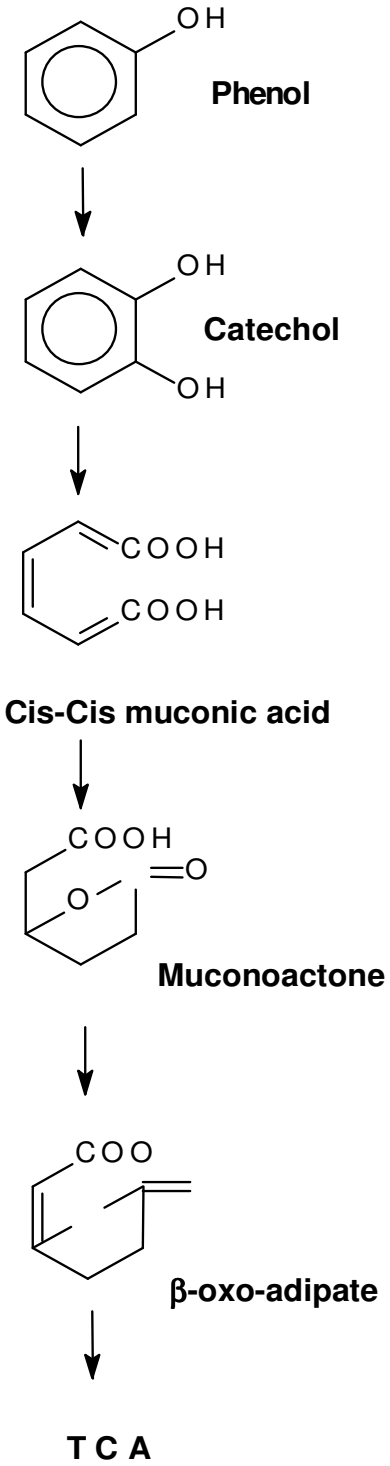

Figure 3. Ortho pathway of phenol degradation.

compounds such as phenol or benzoate via an ortho cleavage pathway using catechol 1,2 dioxygenase (An et al., 2001). These dioxygenases are highly labile enzymes and there requires a detailed investigation into its structural properties. A bacterial strain, *Serratia plymuthica* was able to tolerate phenol up to a concentration of 1050 mg/L. Phenol was degraded through ortho pathway and the crude extract showed the presence of ring cleaving enzyme catechol 1, 2-dioxygenase (Nilotpala and Ingle, 2007).

Catechols are cleaved either by ortho-fission (intradiol, that is, carbon bond between two hydroxyl groups or by a meta-fission (extra diol, that is, between one of the hydroxyl groups and a non-hydroxylated carbon) as given in Figures 2 and 3. Thus the ring is opened and the open ring is degraded (Cerniglia, 1984). As a general rule, most of the halo aromatics are degraded through the formation of the respective halocatechols, the ring fission of which takes place via ortho-mode. On the other hand, most of the non halogenated aromatic compounds 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 would be 2-hydroxy muconic semialdehyde and the products of both ortho and meta pathways are further metabolized as intermediates of TCA cycle. Ortho-pathway is the most productive pathway for the organism as it involves less expenditure of energy.

Phenol hydroxylase (E. C 14. 1.3.7) catalyses the degradation of phenol via two different pathways initiated either by ortho or meta cleavage. There are many reports on phenol hydroxylase and catechol 2, 3 dioxygenase involved in the biodegradation of phenol (Leonard and Lindley, 1999). Hublik and Schinner (2000) reported the characterization of laccase from *Penurious ostreatus*. The enzyme was purified to homogeneity and was characterized. It was a monomeric protein with a molecular weight of 67 KD and with an isoelectric point of 3.6. They observed that the laccase retained most of its activity in high ionic buffer, pH.10, 20°C temperature in the presence of 10 mM benzoic acid and with 35% ethylene glycol. The degradation of phenolic compounds by immobilised laccase from *Streptomyces psammoticus* was evaluated and confirmed by thin layer chromato-graphy and nuclear magnetic resonance spectroscopy (Niladevi and Prema, 2007).

Polyphenol oxidase is a (EC 1.14.18.1) monoxygenase which catalyses the O-hydroxylation of phenols and the oxidation of O-dihydric phenols to O-quinones using molecular oxygen. Laccase are phenol oxidases which utilize molecular oxygen. They are known to have the ability to oxidize polyphenols, meta substituted phenols, diamines and a variety of other components (Kadhim, 1999). The mechanism by which polyphenol oxidase catalyses the conversion of monophenols to O-quinones involves the hydroxylation of monophenols followed by dehydrogenation to form O-quinones. These quinones undergo spontaneous nonenzymatic polymerization in water, eventually forming water insoluble polymers which can be separated from water by filtration (Edwards et al., 1999).

There were various reports on the exploitation of polyphenol oxidase in the detoxification of the phenols. The interest in polyphenol oxidase had been fueled by their potential uses in detoxification of environmental pollutants (Bollag et al., 1988). Production of useful chemicals from lignin (Burton et al., 1993) by polyphenol oxidase was also reported. Garzillo et al. (1998) reported a polyphenol oxidase from the white rot fungus *Trametes trogii*. It was an enzyme with molecular weight 70 KD. The purified enzyme oxidised a number of phenolic compounds. This multicopper oxidases had a wide range of substrate specificity. *Coprinus macrorhizus* and *Arthromyces ramosus* were proved to be effective in removing phenol and phenolic compounds from water (Wu et al., 1998). Of the various enzymes acting on phenol, polyphenol oxidase was the most important one probably because of its increasing demand in lignin degradation (Garzillo et al., 1998). The non specific nature of the polyphenol oxidase was also discussed by Schneider et al. (1999).

Immobilised polyphenol oxidase on chitosan coated polysulphone capillary membranes were used for improved phenolic effluent bioremediation (Edwards et al., 1999). They also highlighted the removal of quinones and other polymerized products using chitosan. Polyphenol oxidases were widely distributed in many plants and fungal species (Robles et al., 2000). They suggested the possibility of using a polyphenol oxidase producing strain of the hyphomycete *Chalara paradoxa* in the detoxification of olive mill wastewater.

Sakurai et al. (2001) showed that the peroxidase from *Coprinus cinereus* could be used for the removal of Bisphenol. Polymerization of the bisphenol by the enzyme was utilized here. Manophenols in aqueous solution could also be removed by peroxidase catalysed oxidation (Xia et al., 2003).

Certain actinomyces and *Streptomyces* strains could produce tyrosinase enzyme, which oxidized halogen substituted phenols. Peroxidases could catalyse the transformation of phenol and halogenated phenols. Peroxidases such as those from *Arthrobacter* and *Streptomyces* strains were being reported as the phenol degrading enzymes (Fetzner and Lingens, 1994). The peroxidase catalysed polymerization process was proved to be very effective in eliminating phenol and a variety of other aromatic pollutants from waste waters (Ghioureliotis and Nicell, 1999). Peroxidases can act on phenol and other aromatic compounds through oxidative coupling. In presence of hydrogen peroxide two equivalents of phenol are converted by each equivalent of enzyme into highly reactive radical species. Once they are formed, they react with one another to yield phenolic polymers. Tyrosinase catalyzes the oxidation of phenols involving the formation of orthoquinones. The mechanism of the enzymatic action of tyrosinase on various phenols was discussed in detail by Siegbahn (2003).

The mechanism of degradation of an organic compound may be unusual (Jenisch-Anton, 1999). The mechanism of degradation is generally decided by the nature of the organic compound, its solubility, and nature of the organism, type of the enzyme and also by the external factors affecting biodegradation. In some cases, through the action of monooxygenase, aromatic com-pounds may be converted into gentisic acid. The fission of this compound occurs between the hydroxyl and carboxyl groups, that is, meta fission. It has been shown in some cases that chloroaromatic compounds such as 4-chlorobenzoate, 4-chlorophenol and others may get dechlorinated during the hydroxylation resulting in the formation of 4–hydroxy benzoates (4-HBA). This 4 HBA on further hydroxylation will be converted to proto-catechuate acid (3,4-dihydroxy benzoic acid), which may be cleaved either through ortho or meta mode.

Several external factors can limit the rate of biodegradation of organic compounds. These factors may

include temperature, pH, oxygen content and availability, substrate concentration and physical properties of contaminants. Each of these factors should be optimized for the selected organism for the maximum degradation of the organic compound of choice. The optimization of the substrate concentration in phenol biodegradation is particularly important since it inhibits the growth of the organism at higher concentrations.

Since civilization will most probably continue to be accompanied by the production of hazardous waste materials, it is necessary to develop efficient strategies for waste management. Biotechnology for hazardous waste management involves the development of biological systems that catalyse the detoxification, degradation or decontamination of environmental pollutants. In future technologies, microbial systems might be the potential tools to deal with the environmental pollutants.

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