METABOLIC PATHWAYS FOR THE BIODEGRADATION OF PHENOL

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Abstract

Organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health. As they persist in the environment, they are capable of long range transportation, bioaccumulation in human and animal tissue and biomagnifications in food chain. Phenolic compounds are hazardous pollutants that are toxic at relatively low concentration. The use of microbial catalysts in the biodegradation of organic compounds has advanced significantly during the past three decades. 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. This also depends on aerobic and anaerobic conditions. Under aerobic conditions, degradation of phenol was shown to be initiated by oxygenation into catechols as intermediates followed by a ring cleavage at either the ortho or meta position, depending on the type of strain. Aerobically, phenol 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.

Index Terms: *Phenols; Aerobic and Anaerobic biodegradation; Microbial metabolism; Ortho and Meta pathway.*

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1. INTRODUCTION

Environmental pollution is considered as a side effect of modern industrial society. The presence of man-made (anthropogenic) organic compounds in the environment is a very serious public health problem. Soil and water of lakes, rivers and seas are highly contaminated with different toxic compounds such as phenol, ammonia, cyanides, thiocyanate, phenol formaldehyde, acrylo- and aceto-nitrile, mercury, heavy metals. Thirty monoaromatics are on the EPA priority pollutant list and 11 of these compounds are among the top of hundred chemicals on the priority list of hazardous substances published by the Agency for toxic substances and disease registry. Monoaromatic hydrocarbons such as benzene, toluene and phenol are obvious choices for studies on biodegradation. Among these, phenols are considered to be pollutants.

Phenol is a basic structural unit for a variety of synthetic organic compounds (Fig.1). It is a white crystalline solid with molecular weight of 94.14 g/mol and formula of C_6H_5OH (ATSDR, 1989; US Environmental Protection Agency, 1990). It has a very strong odour (acrid odour) with an odour threshold of 0.04 ppm (Amoore and Hautala, 1983) and a sharp burning taste. It is soluble in most organic solvents and its solubility in water is limited at room temperature, however above 68^0C it is entirely water-soluble. It is moderately volatile at room temperature (evaporates more slowly than water) and quite flammable. Phenol is a weak acid and in its ionized form, it is very sensitive to electrophilic substitution reactions and oxidations. Phenol finds its application in the production of phenolic resins, caprolactan and bisphenol A, slimicides, disinfectants, antiseptics and medicinal preparations such as ear and nose drops, mouthwashes and sore throat lozenges (ATSDR, 1989). However, phenol and its derivatives are among the most common water and gaseous pollutants. They are widely distributed either as natural or artificial mono aromatic environmental pollutants owing to their common presence in the waste effluents of many industrial processes such as oil refineries, coke oven plants, steel plants, phenolic resin productions, explosives, textiles,

paint and varnish, rubber reclamation plants, stocking factories, cork production and coffee industries .

Therefore, the unwholesome and environmentally unacceptable pollution effects of the wastes have been reported worldwide (Ruiz-Ordaz *et al*., 2001) and the adverse effects of phenol on health are well documented. Phenol is toxic even at low concentrations and the toxicity of phenols for microbial cells has been investigated. Owing to the toxic nature and consequent health hazard of phenol, the need to remove it from wastewaters and polluted environment is very paramount and several physical, chemical and biological removal or treatment technologies have been employed in this regard However, the physico-chemical removal or treatment technologies have been found to have inherent drawback owing to the tendency to form secondary toxic intermediates and also proven to be costly. The focus is on the development of technology that emphasizes detoxification and degradation of the pollutant. Thus, biological removal or treatment technology has turned out to be a favorable alternative because it produces no toxic end products and it is of low cost. This paper reviews the Enzymes involved in the biodegradation of phenolic compounds(Table 1), Phenol-degrading microorganisms (Table 2), Degradation mechanisms of phenols, Intermediates of phenol degradation and metabolic pathway.

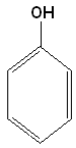

Figure.1 : **Chemical structure of Phenol**

The impacts of pollution on the environment have led to intense scientific investigations. The removal of phenol from industrial effluents has attracted researchers from different fields. The increasing awareness on the environment in both developed and developing countries has initiated more studies of possible solutions for treating phenol.

Table-1: 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	Catechol 2,3 dioxygenase	Ali *et al.* (1998)
5	Phenol	Laccase	Bollag *et al.* (1998)
6	Phenol	Peroxidase	Ghioureliotis and Icell (1998)
7	Phenol	Horse radish peroxidase	Wu *et al.* (1998)
8	Phenol	Catechol 1,2oxygenase	An *et al.* (2001)
9	Bis phenol	Peroxidase	Sakurai *et al.* (2001)
10	Phenol	Tyrosinase	Xiangchun (2003)

Table-2: **Phenol-degrading microorganisms**

Microorganism (Bacteria)	**Reference**
Micrococcus sp.	Tibbles and Baecker , 1989b
Nocardia sp.	Tibbles and Baecker , 1989b
	Vijaygopal and Viruthagiri, 2005
Pseudomonas sp.	Kang and Park, 1997
Pseudomonas cepacia	Arutchelvan *et al*., 2005
*Pseudomonas putida*BH	Soda *et al*., 1998
Pseudomonas putida DSM 548	Monterio *et al*., 2000
Pseudomonas putida EKII	Hinteregger *et al*., 1992
Pseudomonas putida MTCC 1194	Bandhyopadhyaya *et al*., 1998
	Mahadevaswamy *et al*., 2004
Pseudomonas putida Q5	Kotturi *et al*., 1991
	Onsyko *et al*., 2002
Pseudomonas putida NRRL-β -14875	Seker *et al*., 1997

Pseudomonas putida CCRC 14365	Tsuey-Ping Chung,2005
Pseudomonas pictorum NCIM 2077	Sheeja and Murugesan, 2002
Pseudomonas putida ATCC 11172	Loh and Liu, 2001
Pseudomonas putida ATCC 12633	Hughes and Cooper,1996
Pseudomonas putida ATCC 17484	Gonzalez *et al*., 2001a
Pseudomonas putida ATCC 49451	Wang and Loh, 1999
Pseudomonas putida F1 ATCC 700007	Tarik Abu Hamed *et al*., 2003
	Abuhamed *et al*., 2003
Pseudomonas putida ATCC 31800	Gurusamy Annadurai *et al*., 2007
Pseudomonas putida NICM 2174	Annadurai *et al*., 1999
	Annadurai *et al*., 2000
Pseudomonas putida JS6	Spain and Gibson, 1988
Pseudomonas putida F1	Spain and Gibson, 1988
Pseudomonas stutzeri strain SPC2	Ahamad and Kunhi,1996
Pseudomonas testosteroni CPW301	Kim *et al*., 2002
Pseudomonas sp STI	Safia Ahmed,2001
Microorganism (Fungi)	**Reference**
Aspergillus niger	Garcia *et al*., 2000
Aspergillus terreus	Garcia Garcia *et al*., 1997
Coprinus sp.	Guiraud *et al*., 1999
Coprinus cinereus	Masuda *et al*., 2001
C. cinereus	Guiraud *et al*., 1999
C. micaceus	Guiraud *et al*., 1999
Geotrichum candidum	Garcia Garcia *et al*., 1997
Phanerochaete chrysosporium	Garcia *et al*., 2000
Microorganism (Yeast)	**Reference**
Candida maltosa	Ariana Fialova *et al*., 2004
Candida tropicalis	Salmeron- Alcocer *et al*., 2007
Candida tropicalis CHP4	Kumaran, 1980
Candida tropicalis Ct2	Komarkova *et al*., 2003
Candida tropicalis H15	Krug *et al*., 1985
	Krug and Straube, 1986
Microorganism (Algae)	**Reference**
Ankistrodesmus braunii	Gabriele pinto *et al*., 2002
Ochromonas danica	Semple and Cain, 1995
Scenedesmus quadricauda	Gabriele pinto *et al*., 2002

2. MICROBIAL METABOLISM OF PHENOLS

A wide variety of microorganisms are known to be capable of metabolising many of the organic pollutants or chemicals generated and discharged. Metabolic processes are governed by the action of enzymes. Enzymes are specific for each type of reaction. The three major classes of these energy-yielding processes are: aerobic respiration, anaerobic respiration and fermentation. Many microbes are capable of completely metabolising or mineralising different environmental organic pollutants like phenol under aerobic and/or anaerobic conditions and the Pseudomonas species have demonstrated the ability to do this effectively. The wide variety of microorganisms that can aerobically degrade phenol (Table 2) include pure bacterial cultures such as: *Acinebacter calcoaceticus*, *Alcaligenes eutrophus* (Hughes et al., 1984; Leonard and Lindley, 1998), *Bacillus stearothermophilus*, *Pseudomonas cepacia G4* also known as *Burkholderia cepacia G4*, *Pseudomonas picketti*, *Pseudomonas putida* are also capable of degrading phenol.

Amongst all the microorganisms listed as good degraders of phenol, the pure culture of *Pseudomonad*s are the most utilized purposely for metabolic pathway studies and their

ability to utilize or degrade many other aromatic compounds. In *Pseudomonads*, many of its induced enzymes are non-specific and its metabolic pathway contains a high degree of convergence, allow for the efficient utilization of a wide range of growth substrates while the non specificity of the induced enzymes allows for the simultaneous utilization of several similar substrates without an excess of redundant genetic coding for enzyme induction (Hutchinson and Robinson, 1988).

3. DEGRADATION MECHANISM OF PHENOLS

The presence or absence of molecular oxygen plays a crucial role in determining the fate and biodegradation mechanisms of aromatic compounds. In general, phenol can be transformed both under aerobic and anaerobic conditions.

3.1 Aerobic biodegradation of phenol

Figure.2 shows the general metabolic pathway for the biodegradation of phenol. In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation in which the aromatic ring is initially monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to the pre-existing hydroxyl group to form catechol. This is the main intermediate resulting from metabolism of phenol by different microbial strains. Depending on the type of strain, the catechol then undergoes a ring cleavage that can occur either at the ortho position thus initiating the ortho pathway that leads to the formation of succinyl Co-A and acetyl Co-A or at the meta position thus initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde. Leonard and Lindley (1998) have described the biodegradation or metabolism of phenol by *Pseudomonas putida, Pseudomonas cepacia, Pseudomonas picketti* and *Alcaligened eutrophus* respectively via the meta cleavage pathway, while Paller et al. (1995) described the biodegradation of phenol by *Trichosporon cutaneum, Rhodotorula rubura* and *Acinetobacter calcoacetium* respectively via the ortho cleavage pathway.

The meta cleavage pathway for the biodegradation of phenol as presented by Nelson et al. (1987). The mono oxygenase phenol hydroxylase of the *Trichosporon cutaneum, Pseudomonas pickett, Bacillus stearo thermo phylus* BR219 and some species of *acinetobacter* and *alcaligenes* are monocomponent flavoproteins (Kim and Oriel, 1995; Neujahr and Gaal, 1973), while the mono oxygenase phenol hydroxylase of *pseudomonas* CF600 and *Acinetobacter radioresistens* (Shingler et al., 1989) are multicomponent proteins. Multicomponent aromatic mono oxygenases contain at least two components. The former is an oligomeric protein while the latter is a monomeric iron transfer flavoprotein. In fact, the three-component toluene dioxygenase (TDO) from *Pseudomonas putida* uses dioxygenation followed by water elimination to convert phenol to catechol (Spain et al., 1989).

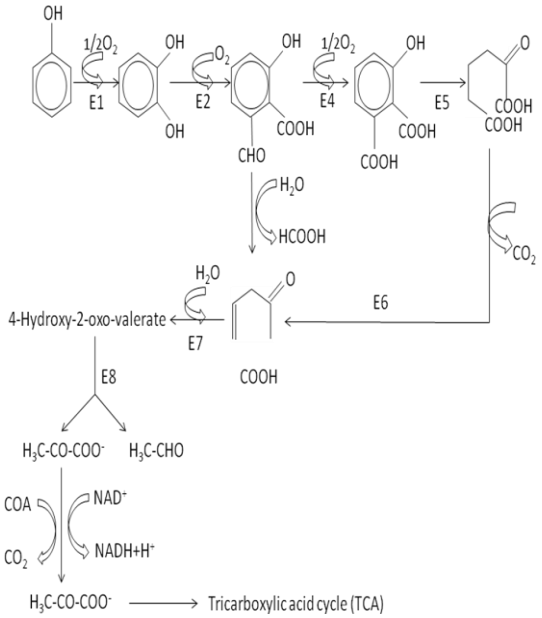

Figure 2: The general metabolic pathway for the biodegradation of phenol

A: Phenol, B: Catechol, C: 2-hydroxymuconic semialdehyde, D: 2-hydroxymuconate,E: 2- oxo- 4- enoadipate, F: 2- oxo-penta-4-enoate, G: Pyruvate, H: Acetaldehyde,I: Acetyl Co A E1: Monooxygenase phenol hydroxylase, E2: Catechol-2, 3-dioxygenase,E3: Hydrolase, E4: Dehydrogenase, E5: Isomerase, E6: Decarboxylase, E7: Hydrotase,E8: Aldose.

3.2 Anaerobic biodegradation of phenol

Phenol can also be degraded in the absence of oxygen and it is less advanced than the aerobic process (Fig.3). It is based on the analogy with the anaerobic benzoate pathway proposed for *Paracoccus denitrificans*in (Williams and Evans, 1975). In this pathway phenol is carboxylated in the para position to 4 hydroxybenzoate which is the first step in the anaerobic pathway. Here the enzyme involved is the 4-hydroxy benezoate carboxylase. The anaerobic degradation of several other aromatic compounds has been shown to include a carboxylation reaction. Carboxylation of the aromatic ring in para position to the hydroxyl group of o-cresol resulting in 3-methyl 4-hydroxybenzoate has been reported for a denitrifying *Paracoccus* like organisms, as well as methogenic consortium was later shown to travel a varity of phenolic compounds including o-cresol, catechol and ortho halogenated phenols via para carboxylation followed by dehydroxylation. The organisms capable of degrading phenol under anaerobic

conditions were *Thauera aromatica* and *Desulphobacterium phenolicum.*

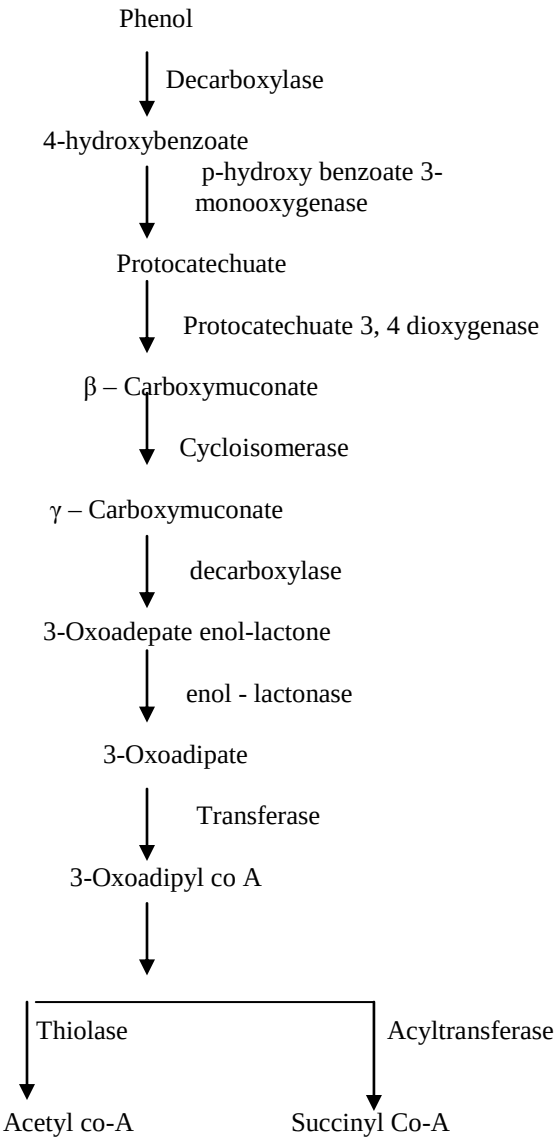

Figure : 3. Flow chart of anaerobic degradation pathway for phenol

4. INTERMEDIATES OF PHENOL DEGRADATION AND METABOLIC PATHWAY

Phenol is converted by bacteria under aerobic conditions to carbon dioxide (Aquino *et al*., 1988), or methane (Fedorak *et al*., 1986). The intermediates in the biodegradation of phenol are benzoate, catechol, cis, cis- muconate, β-ketoadipate, succinate and acetate (Knoll and Winter, 1987). Phenol degradation by microbial pure and mixed cultures have been actively studied (Ahamad, 1995; Chang *et al*., 1998). Most studies on phenol degradation have been carried out with bacteria mainly from the *Pseudomonas* genus (Ahamad , 1995).

Phenol may be degraded in its free form as well as after adsorption onto soil or sediment, although the presence of sorbent reduces the rate of biodegradation. When phenol is the only carbon source, it can be degraded in a bio-film with first-order kinetics at concentrations below 20μg/L at 10ºC. The first-order rates constant are 3 to 30 times higher than those of easily degraded organic compounds and 100-1000 fold at higher concentrations. Howard (1989) reported that phenol degradation rates suggest rapid aerobic degradation in sewage (typically 905 with an 8 h retention time), soil (typically complete biodegradation in 2-5 days), fresh water (typically biodegradation in <1 day), and sea water (typically 50% in 9 days). Anaerobic biodegradation is slower. In bacteria, aromatic compounds are converted to few substrates: catechol, protocatechuate and more rarely gentisate.

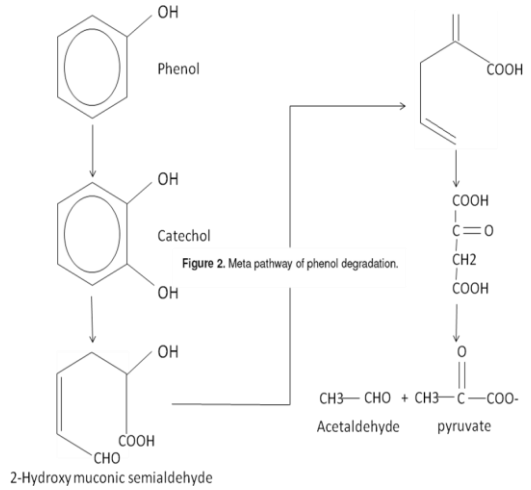

Figure 4: Meta pathway of phenol degradation

The number of bacteria capable of utilizing phenol is only a small percentage of the total population present in, for example, a soil sample (Hickman and Novak, 1989). However, a repeated exposure to phenol may result in acclimation as suggested by a number of researchers (Young and Rivera, 1985; Colvin and Rozich, 1986; Shimp and Pfaender, 1987; wiggins and Alexander, 1988; Tibbles and Baecker, 1989a).

Under aerobic condition, oxygen is used as electron acceptor for the transfer of electrons. In the biodegradation of phenol, phenol is the primary substrate and must be made available in order to have biomass active in the biodegradation process. According to Rittmann and Saez (1993) once active biomass is present, any biotransformation reaction can occur, provide the microorganisms possess enzymes for catalyzing the reaction. These enzymes that are involved in the aerobic metabolism of aromatic compounds usually define the range of substrates that can be transformed by certain metabolic pathway (Pieper and Reineke, 2000).

The first step in aerobic metabolism (Fig. 4) is phenol hydroxylation to catechol by phenol hydroxylase (EC 1.14.13.7) a NADPH-dependent flavoprotein (Neujahr and Gaal, 1973; Enroth *et al*., 1998). It incorporates one oxygen atom of molecular into the aromatic ring to form catechol. Phenol hydroxylases, strictly dependent on the presence of NADPH, have been described in extracts of *T.cutaenum (*Neujhar and Gaal, 1973) and *C. tropicalis* (Neujhar *et al*., 1974). The second step is catalyzed by catechol 1,2-diooxygenase (EC 1.13.11.1; ortho fission) or catechol 2,3-dioxygenase (EC 1.13.11.2; meta fission). After several subsequent steps, the products are incorporated into the Tricarboxylic acid cycle (TCA) or Krebs cycle (Shingler, 1996). It has been established that the aerobic degradation of phenolic compounds is metabolized by different strains through either the ortho- or the meta- cleavage pathway (Bayly and Barbour, 1984; Ahamad and Kunhi, 1996; Shingler, 1996).

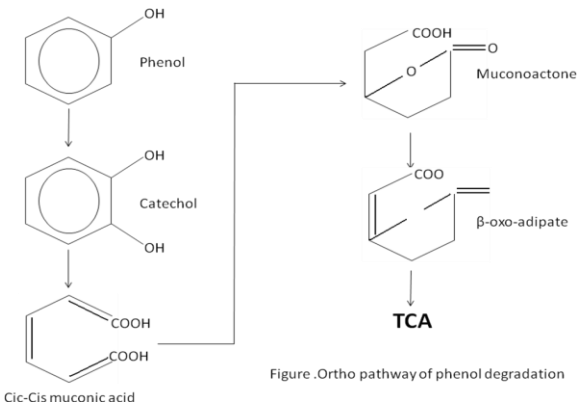

Figure 5: Ortho pathway of phenol degradation

The fission product of ortho-cleavage (Fig.5) 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 semi-aldehyde 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.

5. CONCLUSION

Environmental pollution is one of the major issues, Phenol has become one of the major industrial effluents and the degradation has become a hectic problem. For the degradation of phenol many physical and chemical methods have been in use at present. An alternative method for degradation is biological process or "Bioremediation". Relatively cheaper and effective process and releases lesser harmful products. For the degradation of phenol two methods are followed: aerobic and anaerobic processes. In the aerobic process the end product is carbon dioxide and in anaerobic process the end product is methane or carbon dioxide. Two types of metabolic reactions can take place in the aerobic degradation step meta and ortho degradation pathways. Many microbes are capable of completely metabolizing different environmental organic pollutants like phenol under aerobic and/or anaerobic conditions and the Pseudomonas species have demonstrated the ability to do this effectively

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