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A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS

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ABSTRACT

The environment, as a consequence of industrial and agricultural revolutions, tends to harden with potentially carcinogenic and mutagenic halogen-substituted aromatic compounds. Phenol and its higher molecular homologues are dangerous environmental pollutants. Due to their toxic character, these molecules tend to accumulate in water and soil after being discharged without an adequate treatment. Physical and chemical methods have been designed to remove phenol from effluents but many of these methods are commercially impractical either because of their high operating costs or because of the difficulty encountered in treating the solid wastes generated. In recent years, Biodegradation has been studied as an alternative technology, one of the most efficient and cost effective waste treatment technologies available to industries. Treatment of polluted sites or waste streams can be performed by using systems, in which the number of desirable microorganisms increase because they proliferate at the expense of contaminants. In the present work, detailed description of the properties, sources, hazards, physico-chemical methods, microbial degradation, phenol degrading microorganisms, degradation methods, metabolic pathway and analysis are presented. It has been found that phenol degradation by Pseudomonas putida has been widely adopted as preferred alternative.

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 like chromium, zinc, cadmium, copper, nickel etc. 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.

Chemical identity, physical and chemical properties of phenol

Phenol, C_6H_5OH or hydroxybenzene, is an aromatic molecule containing hydroxyl group attached to the benzene ring structure. Phenol commonly known as carbolic acid (Gardner *et al*. 1978) has a molecular weight of 94.11gm/mol (Lide, 1993). It has a

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melting point of 43°C and forms white to colorless crystals, colorless to pink solid or thick liquid. It has a characteristic acrid smell and a sharp burning taste. Phenol has relatively high water solubility and is soluble in most organic solvents such as aromatic hydrocarbons, alcohols, ketones, ethers, acids, halogenated hydrocarbons (Lide, 1993). However, the solubility is limited in aliphatic solvents. The odour threshold of phenol in air is 0.04 ppm (v/v) (Amoore and Hautala, 1983) and in water between 1 ppm and 7.9 ppm (w/v) (Amoore and Hautala, 1983).

Sources of phenol

The origin of phenol in the environment is from natural, man-made and endogenous sources. Phenol consequent to its manufacture and use in such practices as wood burning, auto exhaust, etc., finds released primarily in air and water. Phenol mainly enters into waters from industrial effluent discharges.

1. Natural Sources: Phenol is a constituent of coal tar, and is formed during decomposition of organic materials. Increased environmental levels may result from forest fires. It has been detected among the volatile components from liquid manure at concentrations of 7-55 µg/Kg dry weight and has an average concentration in manure of 30µg/Kg dry weight.

2. Man-made sources: Man-made sources are from industrial wastes from fossil fuel extraction, wood processing industry, pesticide manufacturing plants (Kumaran and Parachuri, 1997), petroleum refinery, petrochemicals, organic chemical manufacture, coal refining, plastics, pharmaceuticals, tannery, pulp and paper mills (Kumaran & Paruchuri, 1997), as well as from agricultural run-off. Domestic wastewater and chemical spills from several other process industries release phenolic compounds to the environment. (Table 1)

3. Endogenous Sources: An important additional source of phenol may be the formation from various xenobiotics such as benzene (Pekari *et al*. 1992) under the influence of light.

Hazards of phenol

Aromatic hydrocarbons are not as readily biodegradable as the normal and branched. But alkanes, they are somewhat more easily degradable than the alicyclic hydrocarbons. Many of these compounds are toxic and some are known or suspected carcinogens (Sheeja and Murugesan, 2002). The presence of phenol in drinking water and irrigation water represents a serious health hazards to humans, animals, plants and microorganisms.

Phenol concentrations greater than 50ppb are toxic to some form of aquatic life and ingestion of 1gm of phenol can be fatal in human beings (Seetharam and Saville, 2003). Continuous ingestion of phenol for a prolonged period of time causes mouth sore, diarrhea, excretion of dark urine and impaired vision at concentrations levels ranging between 10 and 240 mg/L (Barker *et al*. 1978). Lethal blood concentration for phenol is around 4.7 to 130 mg/100mL. Phenol affects the nervous system and key organs, i.e. spleen, pancreas and kidneys (Manahan, 1994). Phenol is lethal to fish even at relatively low levels, e.g. 5-25 mg/L, depending on the temperature and state of maturity of rainbow trout (Brown *et al*. 1967).

Table 1. Sources of phenol and other related aromatic compounds in wastewater

Sources	Significant phenolic compounds
Petroleum refining sub phe-	Hydrocarbons (alkanes, cycloalkanes, polyaromatic hydrocarbons), benzenes, stituted benzenes, toluenes, n-octanes, n-decanes, napthalenes, biphenyles, nol, cyanide, sulphide and ammonia.
Petrochemicals	Naphtalene, heptanes, benzenes, butadiene. C-4 alcohols, phenol and resorcinol
Basic organic chemical manufacturing	m-amino phenol, resorcinol, dinitrophenol, p-nitrophenol, benzene sulphonic acids, aniline, chlorobenzenes, toluene and resorcinol.
Coal refining matic acid.	Phenol, catechol, o-,m-,p-cresols, resorcinol, hyroquinone, pyrogallol, polyaro- hydrocarbons, pyridine, pycolines, lutidines, xylenes, toulenes, benzoic
Pharmaceuticals	Toluenes, benzyl alcohols, phenyl acetic acid, chlorinates products of benzene, chloroform, ether, ethyl alcohol.
Tannery	Tannin, catechin, phenol, chlorophenol, nitrophenols.
Pulp and Paper mills	Lignin, vanillin, vanillic acid, dehydrodivanillin, ferulic acid, cinnamic acid, synringic acid, vieratric acid, protocatechuic acid, gentisic acid, benzoic acid, guadiachols, catechol, conifery alcohol, dehydrodihydroconiferyl alcohol, phe nyl propionic acid, phenols and chlorophenols.

Phenolic compounds are also responsible for several biological effects, including antibiosis (Gonzalez *et al*. 1990), ovipositional deterrence (Girolami *et al*. 1981) and phytotoxicity (Capasso *et al*. 1992).

Phenol is classified as a priority pollutant owing to their high toxicity and wide spread environmental occurrence. Various regulatory authorities have imposed strict limits to phenol concentration in industrial discharges. Many countries regulate phenol released into the environment. For drinking water, a guideline concentration of 1µg/L (WHO, 1994) has been prescribed. In Malaysia, the Environmental Protection Act, 1974 establishes a phenol concentration of 0.001mg/L for Standard A, 0.1 mg/L for standard B and 5 mg/L other than standard A and B as the limit for wastewater discharges into inland waters. Therefore, it can be seen that disposal of phenol has become a major global concern.

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.

Different treatment methods are available for reduction of phenol content in wastewater. Phenolic wastes are treated by several physico-chemical methods like Chlorination, Advanced oxidation process (Santiago *et al*. 2002), Adsorption, Solvent Extraction, Coagulation, Flocculation, Reverse osmosis, Ozonisation, Photo catalysis and Electrolytic oxidation (Arutchelvan *et al*. 2005).

Chlorination

Chlorine may be applied in gaseous form or as an ionized product of solids. Chlorine can react with naturally occurring organic compounds found in water and produce dangerous compounds, known as disinfection byproducts.

Advanced Oxidation Processes (AOPs)

The AOPs use ozone, UV, ozone in combination with UV (O_3/UV), ozone plus hydrogen peroxide (O_3/H_2O_2), hydrogen peroxide and ultraviolet light (UV/H2O2). The main problem of AOPs lies in the high cost of reagents such as ozone, hydrogen peroxide or energy light sources like ultraviolet lights.

Adsorption

In adsorption process solutes from liquid media are adsorbed onto solids. The most widely used adsorbent for wastewater treatment applications is activated carbon, since it has large internal surface area per unit rate. But its applicability is confined to low concentrations of solutes.

Solvent Extraction

A mixture of two components is treated by a solvent that preferentially dissolves one or more of the components in the mixture. If the initial concentration is less than 2 gm/L, extra operating and capital costs is required.

Coagulation

Coagulation is the formation of small flocs from dispersed colloids using coagulating agents. The major disadvantage of coagulation / flocculation processes is the production of sludge and subsequent separation and removal of it.

Flocculation

Flocculation is the agglomeration of small flocs into larger settleable particles using flocculating agents.

Reverse osmosis

It uses the pressure to drive water through the membrane against the force of osmotic pressure. The main disadvantage is concentration polarization, which is the accumulation of solute molecules on the membrane surface and may cause membrane fouling. Unless membranes are well maintained, algae and other life forms can colonize the membranes.

Ozonisation

The process of treating, impregnating, or combining with ozone. The main disadvantage of this process lies in the high cost of reagents.

Photo catalysis

It is the acceleration of a photoreaction in the presence of a catalyst. The main disadvantage is the additional cost associated with the downstream catalyst separation.

Electrolytic oxidation

A cell containing an electrolyte through which an externally generated electric current is passed by a system of electrodes in order to produce an electrochemical reaction. The main disadvantage is high capital cost.

Hence, the disadvantages like incomplete phenol removal, high reagent and energy requirements,

generation of toxic sludge or other waste products that require careful disposal has made it imperative to look for a cost-effective treatment method that is capable of removing phenol from industrial effluents. As alternatives, slowly biological tools are being substituted in pollution abatement programs. Researchers are studying pollutant degrading microorganisms, which inhabit polluted as well as contaminated environments. This new technology has been loosely grouped together under the term "Bioremediation", a treatment process that uses microorganisms to breakdown, or degrades, hazardous substances into less toxic or non-toxic substances. Harnessing the potential of microbes to degrade phenol has been an area of considerable study to develop Bioremediation approaches, which is considered as "Green Option" for treatment of environmental contaminants.

Microbial degradation

Microbial degradation of chemicals in the environment is a route for their removal. The microbial degradation of these compounds is a complex series of biochemical reactions and often different when different microorganisms are involved. The interdependence of biodegradation, biotransformation and biocatalysis has been reviewed by Parales *et al*. 2002. Microbial degradation of pollutants is crucial in order to predict their longevity and long term effects and also important in the actual remediation process.

Depending on the type of bacteria that are responsible for the degradation i.e., in the presence of free oxygen or oxygen in combined state, bioremediation is classified as "aerobic" or "anaerobic".

In aerobic respiration, oxygen acts as the electron acceptor. Molecular oxygen is a reactant for oxygenase enzymes and is incorporated into the final products. In anaerobic respiration, different inorganic electron acceptors are possible such as NO_3^-, $SO4_2^-$, S^0, CO_2 and Fe_3^+. Most of the biodegradation is aerobic as anaerobic process is relatively slow and is difficult to maintain for bioremediation process. It is preferred where reduction is favored over oxidation as in the case of chlorinated compounds. Many synthetic compounds accumulate in nature because the release rates exceed the rates of microbial and chemical degradation.

Two major reasons have been identified for low degradation rates. First, the biochemical potential to degrade certain compound is limited. This is more likely that fewer chemicals resemble natural compounds. Secondly, the pollutant or other substrates, e.g., appropriate electron acceptors are unavailable to the microflora.

In the natural environment, the rate of degradation can be dependent on physical, chemical and biological factors, which may differ among ecosystems. Alexander 1985, reported that for a microbial transformation to occur, a number of conditions must be satisfied. These include :

1. Microorganisms must exist with the required enzyme to catalyze the specific transformations. There are unspecific enzymes that can attack several types of substrates, while other enzymes can only catalyze the breakdown of one specific bond in a specific compound. Duetz *et al*. 1994, reported that different bacterial strains may also degrade the same compound by different degradation patterns, depending on the types of enzymes used. Many degradation pathways are achieved only by the synergistic relationship of several species (Lappin *et al*. 1985).
2. The chemical must be made available for the microorganism. The inaccessibility may result if the chemical exists in a different phase from that of the bacteria, e.g., in a liquid phase immiscible with water, or sorbed to a solid phase.
3. The success of the degrading strains to proliferate will depend on their ability to compete for the organic compound, oxygen and other environmental factors.

Phenol-degrading microorganisms

Microorganisms that can degrade phenol were isolated as early as 1908 (Evans, 1947). The key components of microbial communities responsible for degradation of phenolic wastes are Pseudomonas species. Their physiological and genetic basis of phenol degradation has been described by many researchers (Kotturi *et al*. 1991; Nurk *et al*. 1991; Kiyohara *et al*. 1992; Motzkus *et al*. 1993; Arquiaga *et al*. 1995; Puhakka *et al*.1995; Buitron and Gonzalez, 1996).

Phenols are metabolized by microorganisms from a variety of different genera and species, as shown in Table 2. Bacteria, fungi, yeast and algae have been reported to be capable of degrading phenol. As shown in Table 2, Pseudomonas putida has been extensively investigated and has been reported to be capable of high rates of phenol degradation (Hutchinson and Robinson, 1988). According to Whitelely *et al*. 2001, isolates that were able to uti-

Table 2. Phenol-degrading microorganisms

Microorganism (Bacteria)	Reference
Acinetobacter sp.	Tibbles and Baecker, 1989a, Oliver *et al*. 2002
Acinetobacter sp. W-17	Usama Beshay *et al*. 2002, Desouky Abd-EI-Haleem *et al*. 2003
A.calcoaceticus AH	Nakamura and Sawada, 2000
A. Johnsonii	Heilbuth *et al*. 2003
Achromobacter sp. E1	Watanabe *et al*. 1996a
Agrobacterium radiobacter	Kowanlska *et al*. 1998
Alcaligenes faecalis	Bastos *et al*. 2000a
Alcaligenes sp. E2	Watanabe *et al*. 1996a
Alcaligenes sp.R5	Watanabe *et al*. 1996a
Alcaligenes strain P5	Baek *et al*. 2001
Arthrobacter sp.	Kar *et al*. 1996
Azoarcus sp.	Shinoda *et al*. 2000
Azospirillium brasilense	Barkovskii *et al*. 1985, Arutchelvan *et al*. 2005
Bacillus brevis	Arutchelvan *et al*. 2006, Balasankar and Nagarajan 2000
B. thermoleovorans A2	Mutzel *et al*. 1996
Burkholderia cepacia G4	Moustafa El-Sayed, 2003
Burkholderia species	Salmeron- Alcocer *et al*. 2007
Comamonas testosterone P15	Yap *et al*. 1999
Comamonas testosterone E23	Yap *et al*. 1999
Halomonas sp.	Hinteregger and Streichsbier, 1997
Halophilic bacteria CA00, CA08, SL03, SL08, SP04	Peyton *et al*. 2002
Iron reducing organism GS-15	Lovley and Lonergan, 1990
Magnetospirillum sp.	Shinoda *et al*. 2000
Micorbacterium phyllospaerae	Salmeron- Alcocer *et al*. 2007
Microorganism (Bacteria)	Reference
Micrococcus sp.	Tibbles and Baecker , 1989b
Nocardia sp.	Tibbles and Baecker , 1989b, Vijaygopal & 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 putid*a 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-ß -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 21812	Daraktchiev *et al*. 1996
Pseudomonas putida ATCC 49451	Wang and Loh, 1999
Pseudomonas putida F1	Reardon *et al*. 2000
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
Ralstonia eutropha	Ozlem Tepe and Arju, 2007, Arzu and Ozlem Tepe, 2005
	Leonard and Lindely, 1999

(Contd.....)

Rhodococcus sp. DCB-p0610	Pai *et al.* 1995
R. erthropolis UPV-I	Prieto *et al.* 2002
Sphingomonas bisphenolicum AO1	Ko-ichi Oshiman *et al.* 2007
Sulfate-reducing bacteria	Boopathy, 1995
Microorganism (Mixed bacterial cultures)	**Reference**
Mixed bacteria	Ha *et al.* 2000
Mixed methanogenic cultures	Karlsson *et al.* 1999
Arthrobacter sp., *Bacillus cereus*	
C. freundii, M. Agilisand P.putida	Kanekar *et al.* 1999
Bacteria + *E.coli* ATCC 33456	Chirwa and Wang, 2000
Clostridium ghonii,	
C. Hastiforme, *C.glycolicum*	Letouneau *et al.* 1995
P.putida F1 and *B.* Strain JS150	Rogers and Reardon, 2000
SRB and AUMB	Boopathy, 1997
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
Coriolus versicolor	Francis Fitz Gibbon *et al.* 1998
Fusarium	Santos and Linardi, 2004, Weijian Cai *et al.* 2007
Graphium LE6, LE11, LA1,LE9, LA5, FIB4, AE2	Santos and Linardi, 2004
Geotrichum candidum	Garcia Garcia *et al.* 1997, Francis Fitz Gibbon *et al.* 1998
Mycelia sterilia	Francis Fitz Gibbon *et al.* 1998
Penicillium AF2,AF4, F1B9	Santos and Linardi, 2004
Pleurotus ostreatus	Fountoulakis *et al.* 2002
Phanerochaete chrysosporium	Garcia *et al.* 2000
	Francis FitzGibbon *et al.* 1998
Rhizobium sp., CCNWTB 701	Gehomg Wei *et al.* 2007
Microorganism (Yeast)	**Reference**
Candida maltosa	Ariana Fialova *et al.* 2004
Candida tropicalis	Salmeron- Alcocer *et al.* 2007, Bastos *et al.* 2000a, Yan *et al.*2005
	Yan Jiang *et al.* 2007, Klein *et al.* 1979, Neujahr *et al.* 1973
	Stephenson, 1990
	Chai *et al.* 2004
Candida tropicalis CHP4	Kumaran, 1980
Candida tropicalis Ct2	Komarkova *et al.* 2003,
Candida tropicalis H15	Krug *et al.* 1985, Krug & Straube, 1986
Candida tropicalis NCYC 1503	Chen *et al.* 2002
Candida tropicalis 708	Shimizu *et al.* 1973
Candida tropicalis YMEC 14	Ettayebi *et al.* 2003
Dabaromyces subglobosus	Chai *et al.* 2004
Rhodotorula glutinis ATCC 28052	Katayama-Hirayama *et al.* 1994
Rhizobium sp., CCNWTB 701	Gehomg Wei *et al.* 2007
Trichosporon cutaneum R57	Alexieva *et al.* 2004, Chai *et al.* 2004
Microorganism (Alga)	**Reference**
Ankistrodesmus braunii	Gabriele pinto *et al.* 2002
Ochromonas danica	Semple and Cain, 1995
Scenedesmus quadricauda	Gabriele pinto *et al.* 2002

Table 3. Phenol biodegradation methods

S.No.	Methods	Reference
1.	Batch Fermentation	Garcia Garcia *et al*. 1997, Vijaygopal *et al*. 2005, Arutchelvan *et al*. 2006 and Balasankar and Nagarajan, 2000.
2.	Continuous culture Fermentation	Salmeron-Alcocer *et al*. 2007
3.	Fed-batch Fermentation	Piakong Bim Mohd, 2006
4.	Packed-bed Fermentation	Ozlem Tepe and Arzu, 2007, Tziotzios *et al*. 2007, Kavitha and Shaik Khasim Beebi, 2003
5.	Fluidized bed Fermentation	Venu Vinod and Venkata Reddy, 2005
6.	Activated sludge process	Gabriela Vazquez-Rodriguez *et al.* 2006.
7.	Lagooning	Orupold *et al*. 2000
8.	Sandstone aquifer	Mette *et al*. 2000.
9.	Immobilization	Arzu and Ozlem, 2005, Desouky Abd- Ei-Haleem *et al*. 2003 and Kowalska *et al*. 1998.
10.	Membrane process	Jolanta Bohdiewicz, 1998, Tsuey-Ping Chung Fermentation 2005 and Bhat *et al*. 2006.
11.	Trickling packed-bed Fermentation	Ehlers and Rose, 2004.

lize phenol as a sole carbon source predominantly belonged to Pseudomonas pseudoalcaligenes. The earlier reports on the decomposition of phenolic compounds by yeasts were by strains belonging to the genera Oospora, Saccharomyces, Candida, Debaryomyces and Trichosporon cutaneum (Harris and Rickettes, 1962; Henderson, 1961; Neujahr and Varga, 1970; Neujahr *et al*. 1974; Hashimota, 1973). Among the yeast strains, Candida tropicalis has been the most studied and able to degrade phenol, phenol derivatives and aliphatic compounds at a relatively high phenol concentration (Krug *et al*. 1985; Chang *et al*. 1995; Ruiz-Ordaz *et al*. 2000). According to Yap *et al*. 1999, mutant strains Comamonas teststeroni E23 has been regarded as the best phenol degrader of all phenol degrading strains reported upto date.

Intermediates of phenol biodegradation and metabolic pathway

Phenol is converted by bacteria under aerobic conditions to carbon dioxide (Aquino *et al*. 1988) and under anaerobic conditions to carbon dioxide (Tschech and Fuchs 1987) or methane (Fedorak *et a*l. 1986). The intermediates in the biodegradation of phenol are benzoate, catechol, cis, cis- muconate, ß-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 of the cultures tested are capable of degrading phenol at low concentrations (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 biofilm with first-order kinetics at concentrations below 20µg/L at 10°C. The first-order rate 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 (Baker and Mayfield, 1980).

In bacteria, aromatic compounds are converted to few substrates: catechol, protocatechuate and more rarely gentisate. Representative aromatic compounds that are converted via catechol are shown in Fig. 1.

As mentioned earlier, bacteria play a major role in the degradation of phenol in soil, sediment and water. 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,

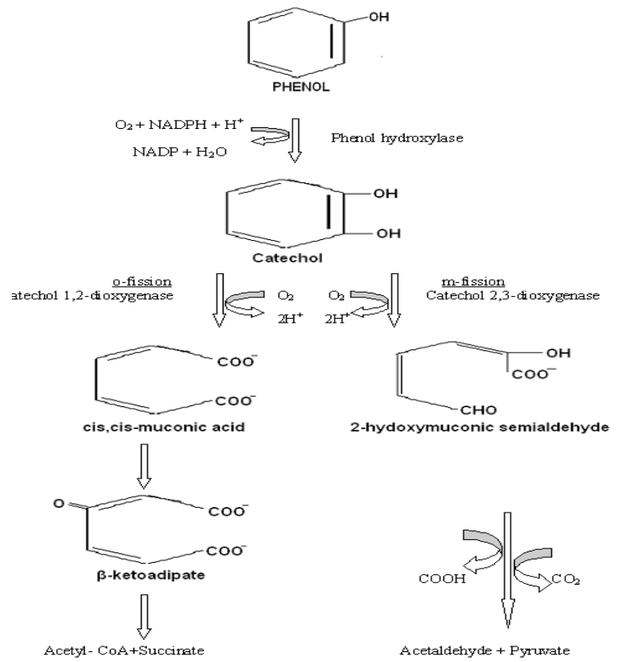

Fig. 1 The main pathways of phenol degradation

1989a). 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.

Phenol may be converted by bacteria by bacteria under aerobic conditions to carbondioxide and under anaerobic conditions to carbon dioxide or methane. The aerobic and anaerobic degradation of phenol has been studied extensively using various microorganisms. (Bak and Widdell, 1986; Karlsson *et al*. 1999; Ruiz-Ordaz *et al*. 2001; Mendoca *et al*. 2004; Yan *et al*. 2005).

Under aerobic condition, oxygen is used as electron acceptor for the transfer of electrons. The transfer of electrons between the electron-donor and electron-acceptor, substrates are essential for creating and maintaining biomass. For instance, 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 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 & Kunhi, 1996; Shingler, 1996).

A number of researchers (Shindo *et al*. 1995; Collins & Dauglis, 1997b; Fan *et al*. 1987; Livingstone and Chase, 1990) suggested that there are many possible biotechnological applications of aromatic-degrading organisms and their constituent enzymes have been investigated including the use in bioreactor systems for removal of toxic waste products or treatment of contaminated wastes. Other applications include the production of valuable biotransformation products such as picolinic acids from catechol (Asano *et al*. 1994), cis, cis-muconic acids from benzoic acid, benzene, toluene or catechol (Choi *et al*. 1997) and also as a reporter gene in diagnostic systems, for example, catechol 2, 3-dioxygenase gene as suggested by Shindo *et al*. (1995).

Sample analysis

Determination of biomass concentration

With samples grown in batch culture, sampling was done periodically to determine the density. Cell density was monitored spectrophotometrically by measuring the absorbance at 600nm using the UV-VIS Spectrophotometer.

The cell dry weight concentration was determined gravimetrically. 5ml aliquots were centrifuged for 15min at 15,000rpm at 10°C in a pre-weighed 30ml tubes. The samples were washed twice with distilled water and the pellets were dried at 105°C in an oven overnight. The difference between the first (empty) and the second weight was used to determine the dry weight of biomass as gm/L.

Dry cell weight was then estimated using calibration curve constructed based on the relationship between optical density at 600nm and dry weight cell.

Determination of specific growth rate

In a batch culture, the exponential increases in biomass after inoculation is measured as a function of time and analyzed to obtained specific growth rate (μ), for that substrate concentration (Yoong and Edgehill, 1993; Yoong *et al*. 2004).

The specific growth rate was measured from the slope of the biomass (dry weight) curve by delineating points between the log growth phase, represented by the equation below:

$$\mu = (\ln Xt - \ln Xo) / t$$

Where Xo = Biomass concentration (dry weight) at time zero.

CONCLUSION

The process of biodegradation is a well-established and powerful technique for treating domestic and industrial effluents. Phenol degradation by Pseudomonas putida has been widely adopted. Many man-made organic compounds are also degraded by microorganism and there is an increase interest in the use of these organisms for pollution control. This paper can be extended by studying the optimization of the process of growth and degradation of phenol by the *P.puitda* using Box-Behnken design experiment, which works on regression analysis of the experimental data collected. The response methodology using the Box-Behnken design of experiments was used to develop a mathematical correlation between the parameters and degradation of phenol. The model predicted has been tested with the support of ANOVA.

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