A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS

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Key words : Phenol, Biodegradation, Bacteria, Fungi, Yeast, Algae, Metabolic pathway.

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, C₆H₅OH 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 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 30 μg/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).

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

<table>
<thead>
<tr>
<th>Sources</th>
<th>Significant phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum refining</td>
<td>Hydrocarbons (alkanes, cycloalkanes, polyaromatic hydrocarbons), benzenes, substituted benzenes, toluenes, n-octanes, n-decanes, naphthalenes, biphenyls, phenol, cyanide, sulphide and ammonia.</td>
</tr>
<tr>
<td>Petrochemicals</td>
<td>Naphtalene, heptanes, benzenes, butadiene. C-4 alcohols, phenol and resorcinol</td>
</tr>
<tr>
<td>Basic organic chemical manufacturing</td>
<td>m-amino phenol, resorcinol, dinitrophenol, p-nitrophenol, benzene sulphonic acids, aniline, chlorobenzenes, toluene and resorcinol.</td>
</tr>
<tr>
<td>Coal refining</td>
<td>Phenol, catechol, o-,m-,p-cresols, resorcinol, hyroquinone, pyrogallol, polyaromatic hydrocarbons, pyridine, pycolines, lutidines, xylene, toluene, benzoic acid.</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Toluene, benzyl alcohols, phenyl acetic acid, chlorinates products of benzenec, chloroform, ether, ethyl alcohol.</td>
</tr>
<tr>
<td>Tannery</td>
<td>Tannin, catechin, phenol, chlorophenol, nitrophenols.</td>
</tr>
<tr>
<td>Pulp and Paper mills</td>
<td>Lignin, vanillin, vanillic acid, dehydrodianillin, ferulic acid, cinnamic acid, syringic acid, vierratic acid, protocatechuic acid, gentisic acid, benzoic acid, guadiachols, catechol, conifer alcohol, dehydrodihydroconiferyl alcohol, phenyl propionic acid, phenols and chlorophenols.</td>
</tr>
</tbody>
</table>
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). 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 1 μg/L (WHO, 1994) has been prescribed. In Malaysia, the Environmental Protection Act, 1974 establishes a phenol concentration of 0.001 mg/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 (O₃/UV), ozone plus hydrogen peroxide (O₃/H₂O₂), 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 NO₃⁻, SO₄²⁻, S⁰, CO₂, and Fe₃⁺. 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. Duez 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; Motzokus et al. 1993; Arquiega 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 Whiteley et al. 2001,
Table 2. Phenol-degrading microorganisms

<table>
<thead>
<tr>
<th>Microorganism (Bacteria)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp.</td>
<td>Tibbles and Baecker, 1989a, Oliver et al. 2002</td>
</tr>
<tr>
<td>Acinetobacter sp. W-17</td>
<td>Usama Beshay et al. 2002, Desouky Abd-El-Haleem et al. 2003</td>
</tr>
<tr>
<td>A. calcoaceticus AH</td>
<td>Nakamura and Sawada, 2000</td>
</tr>
<tr>
<td>A. Johnsonii</td>
<td>Heilbuth et al. 2003</td>
</tr>
<tr>
<td>Achromobacter sp. E1</td>
<td>Watanabe et al. 1996a</td>
</tr>
<tr>
<td>Agrobacterium radiobacter</td>
<td>Kownalska et al. 1998</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>Bastos et al. 2000a</td>
</tr>
<tr>
<td>Alcaligenes sp. E2</td>
<td>Watanabe et al. 1996a</td>
</tr>
<tr>
<td>Alcaligenes sp. R5</td>
<td>Watanabe et al. 1996a</td>
</tr>
<tr>
<td>Alcaligenes strain P5</td>
<td>Baek et al. 2001</td>
</tr>
<tr>
<td>Arthrobacter sp.</td>
<td>Kar et al. 1996</td>
</tr>
<tr>
<td>Azospirillum brasilense</td>
<td>Shinoda et al. 2000</td>
</tr>
<tr>
<td>Bacillus brevis</td>
<td>Barkovskii et al. 1985, Arutchelvan et al. 2005</td>
</tr>
<tr>
<td>B. thermoleovorans A2</td>
<td>Arutchelvan et al. 2006, Balasankar and Nagarajan 2000</td>
</tr>
<tr>
<td>Burkholderia cepacia G4</td>
<td>Mutzel et al. 1996</td>
</tr>
<tr>
<td>Burkholderia species</td>
<td>Moustafa El-Sayed, 2003</td>
</tr>
<tr>
<td>Comamonas testosterone P15</td>
<td>Salmeron- Alcocer et al. 2007</td>
</tr>
<tr>
<td>Comamonas testosterone E23</td>
<td>Yap et al. 1999</td>
</tr>
<tr>
<td>Halomonas sp.</td>
<td>Yap et al. 1999</td>
</tr>
<tr>
<td>Halophilic bacteria C00, C08, SL03, SL08, SP04</td>
<td>Hinteregger and Streichsbier, 1997</td>
</tr>
<tr>
<td>Iron reducing organism GS-15</td>
<td>Peyton et al. 2002</td>
</tr>
<tr>
<td>Magnetospirillum sp.</td>
<td>Lovley and Lonergan, 1990</td>
</tr>
<tr>
<td>Microbacterium phyllosphaerae</td>
<td>Shinoda et al. 2000</td>
</tr>
<tr>
<td>Microorganism (Bacteria)</td>
<td>Salmeron-Alcocer et al. 2007</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>Reference</td>
</tr>
<tr>
<td>Nocardia sp.</td>
<td>Tibbles and Baecker , 1989b, Vijaygopal &amp; Viruthagiri, 2005</td>
</tr>
<tr>
<td>Pseudomonas cepacia</td>
<td>Arutchelvan et al. 2005</td>
</tr>
<tr>
<td>Pseudomonas putidaBH</td>
<td>Soda et al. 1998</td>
</tr>
<tr>
<td>Pseudomonas putida DSM 548</td>
<td>Monterio et al. 2000</td>
</tr>
<tr>
<td>Pseudomonas putida EKII</td>
<td>Hinteregger et al. 1992</td>
</tr>
<tr>
<td>Pseudomonas putida MTCC 1194</td>
<td>Bandhyopadhyaya et al. 1998, Mahadevaswamy et al. 2004</td>
</tr>
<tr>
<td>Pseudomonas putida Q5</td>
<td>Kotturi et al. 1991, Onsyko et al. 2002</td>
</tr>
<tr>
<td>Pseudomonas putida NRRL-8-14875</td>
<td>Seker et al. 1997</td>
</tr>
<tr>
<td>Pseudomonas putida CCRC 14365</td>
<td>Tsuey-Ping Chung,2005</td>
</tr>
<tr>
<td>Pseudomonas pictorium NCIM 2077</td>
<td>Sheeja and Murugesan, 2002</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 11172</td>
<td>Loh and Lui, 2001</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 12633</td>
<td>Hughes and Cooper,1996</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 17484</td>
<td>Gonzalez et al. 2001a</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 21812</td>
<td>Daraktchiev et al. 1996</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 49451</td>
<td>Wang and Loh, 1999</td>
</tr>
<tr>
<td>Pseudomonas putida F1</td>
<td>Reardon et al. 2000</td>
</tr>
<tr>
<td>Pseudomonas putida F1 ATCC 700007</td>
<td>Tarik Abu Hamed et al. 2003, Abuhamed et al. 2003</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 31800</td>
<td>Gurusamy Annadurai et al. 2007</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 31800</td>
<td>Spain and Gibson, 1988</td>
</tr>
<tr>
<td>Pseudomonas putida IS6</td>
<td>Spain and Gibson, 1988</td>
</tr>
<tr>
<td>Pseudomonas putida F1</td>
<td>Spain and Gibson, 1988</td>
</tr>
<tr>
<td>Pseudomonas stutzeri strain SPC2</td>
<td>Ahamad and Kunhi,1996</td>
</tr>
<tr>
<td>Pseudomonas testosteroni CPW301</td>
<td>Kim et al. 2002</td>
</tr>
<tr>
<td>Pseudomonas sp. STI</td>
<td>Safia Ahmed, 2001</td>
</tr>
<tr>
<td>Ralstonia eutropha</td>
<td>Ozlem Tepe and Arju, 2007, Arzu and Ozlem Tepe, 2005</td>
</tr>
<tr>
<td></td>
<td>Leonard and Lindely, 1999</td>
</tr>
</tbody>
</table>

(Contd.....)
Rhodococcus sp. DCB-p0610  
*R. erythropolis* UPV-I  
*Sphingomonas bisphenolicum* AO1  
Sulfate-reducing bacteria

**Microorganism (Mixed bacterial cultures)**

Mixed bacteria  
Mixed methanogenic cultures  
*Arthrobacter* sp., *Bacillus cereus*  
*C. freundii, M. Agilisand P. putida*  
Bacteria + *E. coli* ATCC 33456  
Clostridium ghoni,  
*C. Hastiforme, C. glycolicum*  
P. putida F1 and B. Strain JS150  
SRB and AUMB

**Microorganism (Fungi)**

*Aspergillus niger*  
*Aspergillus terreus*  
*Cepnitus sp.*  
*C. cinereus*  
*C. micaceus*  
*Ceriolus versicolor*  
Fusarium  
*Graphium* LE6, LE11, LA1, LE9, LA5, FIB4, AE2  
*Geotrichum candidum*  
*Mycelia sterilia*  
*Penicillium AF2,AF4, FIB9*  
*Pleurotus ostreatus*  
*Phanerochaete chrysosporium*  
*Rhizobium sp., CCNWTB 701*

**Microorganism (Yeast)**

*Candida maltosa*  
*Candida tropicalis*

*Candida tropicalis CHP4*  
*Candida tropicalis C12*  
*Candida tropicalis H15*  
*Candida tropicalis NCCY 1503*  
*Candida tropicalis 708*  
*Candida tropicalis YM4*  
*Dabaromyces subglobosus*  
*Rhodotorula glutinis ATCC 28052*  
*Rhizobium sp., CCNWTB 701*  
*Trichosporon cutaneum R57*

**Microorganism (Alga)**

*Ankistrodesmus braunii*  
*Ochromonas danica*  
*Scenedesmus quadricauda*
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Table 3. Phenol biodegradation methods

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Continuous culture Fermentation</td>
<td>Salmeron-Alcocer et al. 2007</td>
</tr>
<tr>
<td>5.</td>
<td>Fluidized bed Fermentation</td>
<td>Venu Vinod and Venkata Reddy, 2005</td>
</tr>
<tr>
<td>7.</td>
<td>Lagooning</td>
<td>Orupold et al. 2000</td>
</tr>
</tbody>
</table>

isolates that were able to utilize 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 up to 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 (Fedork 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 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 Baeccker,
Fig. 1 The main pathways of phenol degradation

1989a). Phenol may be degraded in its free form as well as after sorption onto soil or sediment, although the presence of sorbent reduces the rate of biodegradation.

Phenol may be converted by bacteria under aerobic conditions to carbon dioxide 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.cutaneous (Neujhar and Gaal, 1973) and C. tropicalis (Neujhar et al. 1974). The second step is catalyzed by catechol 1,2-dioxygenase (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
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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 10ºC in a pre-weighed 30ml tubes. The samples were washed twice with distilled water and the pellets were dried at 105ºC 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 ($\mu$), 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:

$$\mu = (\ln X_t - \ln X_0) / t$$

Where $X_0$ = 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 microorganisms 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. putida 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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