A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS

M.V.V.CHANDANA LAKSHMI AND V. SRIDEVI

Department of chemical Engineering (Biotechnology), College of Engineering, Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India

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, 1995). 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 (Kumar and Parachuri, 1997), petroleum refinery, petrochemicals, organic chemical manufacture, coal tar, Phenol, cyanide, sulphide and ammonia.

Phenol concentrations greater than 50µg/l are toxic to some form of aquatic life and ingestion of ligm 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, 2003). Lethal blood concentration for phenol is around 4.7 to 130 mg/L (Barker et al, 1978). 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).

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 0.01mg/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 processes (Dias et al, 2002). Adsorption, Solvent Extraction, Coagulation, Flocculation, Reverse osmosis, Ozonisation, Photo catalysis and Electrolytic oxidation (Aratuchelvan 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 larger internal surface area per unit rate. But its applicability is confined to low concentrations of solutes.

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 of coagulation / flocculation processes is the production of sludge and subsequent separation and removal of it.

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,
A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFlUENTS

Microbial degradation of chemicals in the environment is a route for their removal. The 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 oxygen-gaze 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 depend 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 transformation. 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 embedded 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. 1995; Arquiaga et al. 1996; Puahakka 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, isolates that were able to utilize

<table>
<thead>
<tr>
<th>Microorganism (Bacteria)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp.</td>
<td>Tibbles and Bascker, 1999a, Oliver et al. 2002</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>Kovarlika 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. E3</td>
<td>Bask et al. 2001</td>
</tr>
<tr>
<td>A. xyreus</td>
<td>Kat et al. 1996</td>
</tr>
<tr>
<td>Aspergillus brasiliense</td>
<td>Shinoda et al. 2000</td>
</tr>
<tr>
<td>Bacillus brevis</td>
<td>Barkovski et al. 1985, Arutchelvan et al. 2005</td>
</tr>
<tr>
<td>B. thermoleovorans A2</td>
<td>Arutchelvan et al. 2005, 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 P11</td>
<td>Salmeron-Aloece et al. 2007</td>
</tr>
<tr>
<td>Comamonas testosterone E23</td>
<td>Yap et al. 1999</td>
</tr>
<tr>
<td>Halomonas sp.</td>
<td>Hinterreger and Streichhuet, 1997</td>
</tr>
<tr>
<td>Halophilic bacteria CAO0, CAO8, SL03, SL08, SP04</td>
<td>Poyton et al. 2002</td>
</tr>
<tr>
<td>Iron reducing organism GS-15</td>
<td>Lovley and Lonergan, 1990</td>
</tr>
<tr>
<td>Magnetsporium sp.</td>
<td>Shinoda et al. 2000</td>
</tr>
<tr>
<td>Microbacterium phyllospaera</td>
<td>Salmeron-Aloece et al. 2007</td>
</tr>
<tr>
<td>Microorganism (Bacteria)</td>
<td>Reference</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>Reference</td>
</tr>
<tr>
<td>Nocardia sp.</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas cepacia</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putidaATCC 1194</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida DSM 548</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida EGB1</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 21812</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 4951</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 17484</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 23182</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 12633</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida F1</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida F1 ATCC 700007</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 31800</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 2174</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 21802</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida ATCC 6591</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas putida strain SPC2</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas testosterone CPW301</td>
<td>Reference</td>
</tr>
<tr>
<td>Pseudomonas sp. ST1</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia eutropha</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia eutropha CPW301</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. CA00, CA08, SL03, SL08, SP04</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. E1</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. E2</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Hinteregger and Streichsbier, 1997</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Kar</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Kowanlska</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. MTCC 1194</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. NCIM 2077</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Q5</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Shikami</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Shinoda</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Tibbles and Baecker, 1989b</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Vijaygopal &amp; Virutha, 2005</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
<tr>
<td>Ralstonia sp. Wang and Loh, 1999</td>
<td>Reference</td>
</tr>
</tbody>
</table>
A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS

Chandana Lakshmi and Sridevi

Rhodococcus sp. DCB-p0610
R. orthosporus LIPV-1
Sphingomonas bisphosphonicum AO1
Sulfate-reducing bacteria

Microorganism (Mixed bacterial cultures)
Mixed bacteria
Mixed methanogenic cultures
Arthrobacter sp., Bacillus cereus
C. freundii, M. Agilis
P.putida F1 and R. Strain JS350
SRB and AUMB

Microorganism (Mixed bacterial cultures)
Mixed bacteria
Mixed methanogenic cultures
Arthrobacter sp., Bacillus cereus
C. freundii, M. Agilis
P.putida F1 and R. Strain JS350
SRB and AUMB

Microorganism (Fungi)

Aspergillus niger
Aspergillus terreus
Cephrus sp.
Cephrus cinereus
C. micrurus
C. versicolor
Fusarium
Graphium L16, LE11, LA1, LE9, LA5, FIB4, AE2
Geotrichum candidium
Mycelia sterilia
Penicillium AF2, AF4, F189
Penicillium ochrochloron
Phanerochaete chrysosporium
Rhizobium sp., CCNWBT 701

Microorganism (Yeast)
Candida maltosa
Candida tropicalis

Candida tropicalis CHP4
Candida tropicalis C12
Candida tropicalis H15
Candida tropicalis NCYC 1503
Candida tropicalis 708
Candida tropicalis YMEC 14
Dabouromyces subglobose
Rhodotyrella glutinis ATCC 28052
Rhizobium sp., CCNWBT 701
Trichosporon cutaneum R57

Microorganism (Alga)
Ankistrodesmus braunii
Ochrocronas danica
Scenedesmus quadrauda

Pai et al. 1995
Prieto et al. 2002
Koichi Oshiman et al. 2007
Booapathy, 1995

Reference
Ha et al. 2000
Karlsson et al. 1999
Kanekar et al. 1999
Chirwa and Wang, 2000
Letouneau et al. 1995
Rogers and Bearden, 2000
Booapathy, 1997

Reference
Garica Garcia et al. 1997
Garica Garcia et al. 1999
Masuda et al. 2001
Guitraud et al. 1999
Guitraud et al. 1999
Francis Fitz Gibbon et al. 1998
Santos and Linardi, 2004
Weijian Cai et al. 2007
Santos and Linardi, 2004
Garica Garcia et al. 1997, Francis Fitz Gibbon et al. 1998
Francis Fitz Gibbon et al. 1998
Santos and Linardi, 2004
Fountoulakis et al. 2002
Garica Garcia et al. 2000
Francis Fitz Gibbon et al. 1998
Geohong Wei et al. 2007

Reference
Artina Fiallova et al. 2004
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
Kumaran, 1980
Komarkova et al. 2003,
Krug et al. 1985, Krug & Straube, 1986
Chen et al. 2002
Shimizu et al. 1973
Eittayeb et al. 2003
Chai et al. 2004
Katayama-Hirayama et al. 1994
Geohong Wei et al. 2007
Alexeiva et al. 2004, Chai et al. 2004

Reference
Gabriele pinto et al. 2002
Semple and Cain, 1995
Gabriele pinto et al. 2002

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>

Phenol is a saline carbon source predominantly belonged to Pseudomonas pseudocaligeneales. 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 (Harriss and Ricketts, 1962; Henderson, 1961; Neujahr and Varga, 1970; Neujahr et al. 1974; Hashimoto, 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-Orudaz et al. 2000). According to Yap et al. 1999, mutant strains Comamonas testosteroni 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 (Tiesche and Fuchs 1987) or methane (Fedorak et al. 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 10ºC. The first-order rate constant is 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 90% 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 Pfander, 1987, Wiggins and Alexander, 1988; Tiibbes and Baier, 1989).
A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS

CHANDANA LAKSHMI AND SRIDEVI

Fig. 1 The main pathways of phenol degradation

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 bacterial degradation 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 the degradation of phenol, in the primary substrate and must be made available in order to have biomass active in the biodegradation process. According to Rittmann and Saerz (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 pathways (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. cutaneum (Neujahr 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 oxygenase (EC 1.13.11.1; ortho fission) or catechol 2,3-dioxygenase (EC 1.13.11.2; meta fission) 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. Biodegradation 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 10C in a pre-weighed 30ml tubes. The samples were washed twice with distilled water and the pellets were dried at 105C 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 of biomass.

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 obtain specific growth rate (u), for that substrate concentration. Yoon and Edgehill, 1993; Yoon 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:

\[ \mu = \ln(X_f) - \ln(X_0) \]

Where \[ X_0 = \text{Biomass concentration (dry weight)} \]

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 organics 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.

REFERENCES


A REVIEW ON BIODEGRADATION OF PHENOL FROM INDUSTRIAL EFFLUENTS


Microbiology.


Ko-ichi Oshima, Yuzi Tsutsumi, Tomoaki Nishida and Yoshinobu Matsumura. 2007. Isolation and characterization of a novel bacterium Sphingomonas bisphenolicum strain AO1, that degrades bisphenol A. Biodegradation. 18: 247-255.

Ko-ichi Oshiman, Yuzi Tsutsumi, Tomoaki Nishida and Yoshinobu Matsumura. 2007. Isolation and characterization of a novel bacterium Sphingomonas bisphenolicum strain AO1, that degrades bisphenol A. Biodegradation. 18: 247-255.


Tibbles, B.J. and Baecker, A.A.W. 1989b. Effect of pH and inoculum size on phenol degradation by bacteria isolated from landfill waste. Environmental Pollution. 59 (3) : 227-239.


ECOLOGY, ENVIRONMENT AND CONSERVATION
ISSN-0971-765X
Editor - DR. R.K. TRIVEDY

ECOLOGY, ENVIRONMENT AND CONSERVATION is one of the leading environmental journals from India. It is widely subscribed in India and abroad by individuals in education and research as well as by industries, Govt Departments and Research institutes.

Ecology, Environment and Conservation is abstracted in -

COVERAGE

SCOPE
1. Terrestrial Ecology
2. Aquatic Ecology
3. Forest conservation Pollution
4. Environmental Pollution
5. Soil Conservation
6. Waste Recycling
7. Environment Impact Assessment
8. Hazardous Waste Management
9. Biodiversity
10. Ecotoxicology
11. Environmental Education
12. Waste Management
13. Floristic and Faunistic Studies of Various Ecosystems
14. Radiation Hazards
15. Bioremediation ecosystems
16. Pollution Control
17. Climate change

SUBSCRIPTION RATES
INDIA

<table>
<thead>
<tr>
<th></th>
<th>One year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>1000.00</td>
</tr>
<tr>
<td>Organisation</td>
<td>1800.00</td>
</tr>
</tbody>
</table>

For subscribing the journal, please send the necessary amount by DD/MO in favour of

ENVIRO MEDIA
1, Sheila Apartment, Behind Urban Bank, Rukmini Nagar Branch, Post Box- 90,
KARAD- 415 110, INDIA ; ((91-2164- 220369; 020 27210103;
E-mail : str_rktem@sancharnet.in