

*Review Article*

## Microbial Degradation of Phenol- A Review

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*Received: 02/02/2015**Revised: 18/02/2015**Accepted: 19/02/2015*

### ABSTRACT

Aromatic compounds are widely distributed in nature and free phenols are frequently liberated as metabolic intermediates during the degradation of plant materials. In recent years the natural supply of phenolic substances has been greatly increased due to the release of industrial byproducts into the environment. Effluents from petrochemical, textile and coal industries contain phenolic compounds in very high concentration; therefore there is a necessity to remove phenolic compounds from the environment. Among various techniques available for removal of phenols, biodegradation is an environment friendly and cost effective method. The efficiency of biodegradation of organic compounds is influenced by the type of the organic pollutant, the nature of the organism, the enzyme involved, the mechanism of degradation and the nature of the influencing factors. This also depends on aerobic and anaerobic conditions. Under aerobic conditions, degradation of phenol was shown to be initiated by oxygenation into catechols as intermediates followed by a ring cleavage at either the ortho or meta position, depending on the type of strain. Aerobically, phenol is first converted to catechol, and subsequently, the catechol is degraded via ortho or meta fission to intermediates of central metabolism. This paper describes about the various sources of phenol, various microorganisms involved in the biodegradation including aerobe and anaerobe, effect of environmental parameters on phenol degradation and kinetic analysis of biodegradation.

**Key words:** Phenol, Aerobic and Anaerobic biodegradation, Microorganisms, Reactors, Ortho and Meta pathway, Microbial metabolism.

### INTRODUCTION

Environmental pollution is considered as a side effect of modern industrial society. With the immense growth of industries major problem is encountered as contamination of the environment with hazardous and toxic chemicals. Phenolics, one of the major pollutants, are discharged in the waste water from the various industries such as phenol resin and pharmaceutical, oil refineries, petrochemical plants, ceramic plants, steel plants, and coal

conversion processes. Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds. [1] Phenol and its higher homology are aromatic molecules containing hydroxyl group attached to the benzene ring structure.

The origin of phenol in the environment is both industrial and natural. Phenol pollution is associated with pulp and paper mills, coal mines, refineries, wood preservation, plants and various chemicals

industries as well as their wastewaters. Due to their high inhibitory and antibacterial activity, phenols may create problems in the operation of biological treatment plants. They also add odour to drinking and food processing water and have mutagenic and carcinogenic effects. [2]

Biological processes using microbial systems provide an alternative to the existing physical/ chemical technologies (expensive and commercially unattractive) because they are more cost-effective, environment friendly and do not produce large quantities of sludge. [3] Number of microorganisms can utilize phenol under aerobic conditions as source of carbon and energy. Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO<sub>2</sub>, H<sub>2</sub>O, NO<sub>3</sub> and other inorganic compounds.

Due to the toxic properties, including permeabilisation of cellular membranes and cytoplasm coagulation, phenolic contaminants can damage sensitive cells and cause profound health and environmental problems. [4] The World Health Organization has limited phenol concentration in the water to 1 mg/L Toxicity of phenolic compounds inhibits biological treatment or even eliminates sensitive micro-organisms from biological wastewater treatment process and significantly reduces the biodegradation of the other components. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna. Hence, it is necessary to remove phenol from industrial effluents before discharging them into the environment. Many technologies exist for the treatment of phenols, but their use is limited due to the fear of creating toxic intermediates particularly from non-biological process. Many aerobic bacteria have been confirmed to use aromatic compounds as the sole source of carbon and energy [5] which

suggests using phenol as nutrient to the organism and thereby converts phenol to nontoxic component. The biological treatment of industrial wastewaters usually depends upon the oxidative activities of microorganisms. Filamentous fungi can be an important source of phenol-degrading species as they grow frequently in wood where phenolic structures are present. Nevertheless filamentous fungi are not frequently used due to difficulties in their cultivation in liquid media and their slow growth rate in comparison with most of the other microbial species.

### **Mechanism of biodegradation**

Biodegradation is the process of decaying or reduction of different organic materials and toxic metals to their non toxic form with the help of microorganisms. In this process complete mineralization of the starting compound to simpler ones like CO<sub>2</sub>, H<sub>2</sub>O, NO<sub>3</sub> and other inorganic compounds takes place. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals are the micronutrients that also show a significant impact on degradation behavior is reported. [6]

Most of the efficient phenol degrading microorganisms are capable of using phenol as the sole source of carbon and energy for their growth and metabolism. Microorganisms capable of degrading phenol do so with the action of a variety of enzymes. They first convert Phenol to catechol with the help of hydroxylase enzyme. Catechol is then degraded to its intermediates via ortho or meta cleavage with the help of another set of enzymes. The enzyme catechol 2, 3 dioxygenase cuts the benzene ring of catechol at the meta

position and the enzyme catechol 1, 2 dioxygenase cuts the benzene ring at the ortho position. The intermediates released through ortho and meta cleavage are finally consumed by the microbes with the help of various enzymes through the TCA cycle resulting in CO<sub>2</sub>, metabolites and energy. [7]

Because of widespread occurrence of phenol in the environment many microorganisms utilize phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms. They produce several enzymes like Polyphenol oxidase, Catechol 2,3 dioxygenase, Laccase, Peroxidase, Horse radish peroxidase, Tyrosinase, Catechol 1,2 dioxygenase etc. The major microorganisms are bacteria (*Alcaligenes*, *Arthrobacter*, *Pseudomonas*, *Cyanobacterium*, *Bacillus*) fungi (*Candida*, *Fusarium*, *Graphium*, *Ochromonas*, *Aspergillus*) and yeast (*Phanerochaete*, *Rhodococcus*, *Rhodotorula*, *Sphingomonas*, *Trichosporon*). [8]

### **Aerobic biodegradation of phenol**

In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation in which the aromatic ring is initially monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to the pre-existing hydroxyl group to form catechol. This is the main intermediate resulting from metabolism of phenol by different microbial strains. Depending on the type of strain, the catechol then undergoes a ring cleavage that can occur either at the ortho position thus initiating the ortho pathway that leads to the formation of succinyl Co-A and acetyl Co-A or at the meta position thus initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde. A report described the biodegradation or metabolism of phenol by *Pseudomonas putida*,

*Pseudomonas cepacia*, *Pseudomonas picketti* and *Alcaligenes deutorphus* via the meta cleavage pathway, [9] while another report described the biodegradation of phenol by *Trichosporon cutaneum*, *Rhodotorula rubra* and *Acinetobacter calcoacetium* respectively via the ortho cleavage pathway. [5]

The meta cleavage pathway is one of the methods for the biodegradation of phenol. [10] The mono oxygenase phenol hydroxylase of the *Trichosporon cutaneum*, *Pseudomonas pickett*, *Bacillus stearothermophilus* BR219 and some species of *Acinetobacter* and *Alcaligenes* are monocomponent flavoproteins [11] while the mono oxygenase phenol hydroxylase of *Pseudomonas* CF600 and *Acinetobacter radioresistens* are multicomponent proteins. [12] Multicomponent aromatic mono oxygenases contain at least two components. The former is an oligomeric protein while the latter is a monomeric iron transfer flavoprotein. In fact, the three-component toluene dioxygenase (TDO) from *Pseudomonas putida* uses dioxygenation followed by water elimination to convert phenol to catechol. [13]

### **Anaerobic biodegradation of phenol**

Phenol can also be degraded in the absence of oxygen and it is less advanced than the aerobic process. In this pathway phenol is carboxylated in the para position to 4-hydroxybenzoate which is the first step in the anaerobic pathway. [14] Here the enzyme involved is the 4-hydroxybenzoate carboxylase. The anaerobic degradation of several other aromatic compounds has been shown to include a carboxylation reaction. Carboxylation of the aromatic ring in para position to the hydroxy group of o-cresol resulting in 3-methyl 4-hydroxybenzoate has been reported for a denitrifying *Paracoccus* like organisms, as well as an ethogenic consortium was later shown to travel a

variety of phenolic compounds including o-cresol, catechol and ortho halogenated phenols via paracarboxylation followed by dehydroxylation. [10] The organisms capable of degrading phenol under anaerobic conditions were *Thauera aromatica* and *Desulphobacterium phenolicum*.

The intermediates in the biodegradation of phenol are benzoate, catechol, cis, cis- muconate,  $\beta$ -ketoadipate, succinate and acetate. [15] Phenol degradation by microbial pure and mixed cultures has been actively studied. Most studies on phenol degradation have been carried out with bacteria mainly from the *Pseudomonas* genus. Phenol may be degraded in its free form as well as after adsorption onto soil or sediment, although the presence of sorbent reduces the rate of biodegradation. When phenol is the only carbon source, it can be degraded in a bio-film with first-order kinetics at concentrations below 20 $\mu$ g/L at 10°C. The first-order rates constant are 3 to 30 times higher than those of easily degraded organic compounds and 100-1000 fold at higher concentrations. The 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). [16] Anaerobic biodegradation is slower. In bacteria, aromatic compounds are converted to few substrates: catechol, protocatechuate and more rarely gentisate.

### **Various Microorganisms Involved In Phenol Degradation**

Phenol, an aromatic hydrocarbon is degraded by various microorganisms, which utilizes phenol as the sole carbon source for the growth of the organisms. Among the various microorganisms *Pseudomonas putida* is the most popular organism for the

degradation of phenol as this species uses phenol as the carbon source. It has been reported that the *Pseudomonas* species follows a typical meta cleavage pathway for metabolizing phenol at relatively low concentrations. [17] A number of both aerobic and anaerobic phenol degrading microorganisms have been isolated and characterized, although microorganisms capable of aerobic phenol degradation were described as early as 1908. In addition to bacteria, fungi are known for their diversity and remarkable ability to degrade phenolic compounds. In contrast to bacteria, fungi are able to extend the location of their biomass through hyphal growth. They are able to grow under environmentally stressed conditions such as low nutrient availability, low water activity and at low pH values where bacterial growth might be limited. *Trichosporon cutaneum*, *Candida* species, *Rhodotorula* species were able to utilize phenol as sole source of carbon and energy [18] *Fusarium flocciferum* [19] white rot fungi [20] *Phanerochaete chrysosporium* [21,22] have also been shown to metabolize phenols. In few reports of phenol degradation the diauxic growth is noted during the sequential degradation of 4-methylphenol. [23]

Recent studies indicate that the 4-chlorophenol, 4-nitrophenol and phenol cause adaptive effects in the membrane of *Aspergillus chlorophenolicus*. Degradation of monochlorophenols as sole source of carbon in aerobic batch culture has been examined by mixed microbial community. The influences of supplementary conventional carbon source on enhancing the biotransformation rates of phenol as the primary substrate and 4-chlorophenol as a non growth substrate has been studied by medium augmentation with conventional carbon sources. Parameters such as pollutant concentrations, viable biomass, concentrations, existence of inhibitor,

temperature, pH, microbial completion and microbial adaptation are the most important parameters that affect phenol biodegradation rate depends on the period in which the culture was adapted to phenol.

### **Kinetics of Degradation of Phenol**

Phenol is not easily biodegradable and inhibits the innate activity of most types of microorganisms at higher and lower concentrations. Moreover the contributions to total biodegradation efficiency in mixed autochthonic flora cannot be well described. Therefore metabolic and kinetics studies of pure or exactly defined mixed cultures is necessary for estimating the kinetic parameter of growth and modeling bioprocess running in a suitable type of bioreactor, besides this the performance of biological treatment systems is largely depend on the fundamental understanding of toxic substrate utilization which is essential for defining operational conditions for effective removed compounds during wastewater purification. A variety of factors are known to influence the kinetics of microorganisms including temperature, pH, availability of dissolved oxygen and toxic strength.

#### **Effect of pH**

The internal environment of all living cell is believed to be approximately neutral. Most organisms cannot tolerate pH values below 4.0 or above 9.0. [24] At low (4.0) or high (9.0) pH values acids or bases can penetrate into cells more easily, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells. The optimum for phenol degradation is 7.0 for *Pseudomonas putida* NICM 2174. [25]

#### **Effect of temperature**

Temperature plays an important role than nutrient availability in the degradation of organic pollutants. Various studies proved that phenol biodegradation was significantly

inhibited at 30°C. [26] However, most laboratory studies on phenol degradation have been carried out at an optimum temperature of 30°C and also described that when the temperature increased from 30°C to 34°C no phenol degradation was observed due to cell decay, which shows that the phenol degradation is a temperature dependent process. [27] Growth rates in general roughly double for each 10°C rise in temperature within the usual mesophilic operational range from 10 to 30°C. Growth rates generally do not change between 35°C and 40°C, but denaturation of proteins at higher temperatures slows growth rates for mesophiles. [28] However, different mixed cultures adapted to thermophilic temperatures have optimum temperatures range of 55 to 65°C. Thermophiles do not function well at the intermediate temperature of 40 to 45°C as mesophilic organisms. Thus, one must make the decision to operate at the lower mesophilic range with an optimum temperature of around 35°C or in the thermophilic range with a temperature optimum of 55 to 60°C.

#### **Effect of additional carbon sources on phenol degradation**

Biological degradation of phenol has been studied using various pure and mixed cultures. Several studies have been carried out with the *Pseudomonas putida* in pure cultures [29] in which, phenolics degraded via the meta-pathway. [30] However it has been found that these bacteria suffer from substrate inhibition, whereby growth and consequently phenol degradation is inhibited at high phenol concentrations. [31] Various methods have been proposed to overcome substrate inhibition in order to treat high strength phenolic wastewater. These include adapting the cells to higher phenol concentration immobilization of the cells and using genetically engineered microorganisms. Another possible method increasing the tolerance of the cells to

substrate inhibition is to supplement the growth medium with conventional carbon sources, such as yeast extract or glucose. It has also been noted that the presence of yeast extract enhanced the affinity of *Pseudomonas putida* for phenol. [32]

It was found that the presence of glucose attenuated the rate of phenol removal by phenol consuming cells. [33] Studies on *Pseudomonas aeruginosa* with peptone and glucose as additional nutrients showed highest phenol degradation. The rate of phenol degradation was improved when peptone was supplemented at the concentration between 0.25 and 1.0 g/L, with an optimum of 0.25 g/L. Peptone at low concentration influence the rate of degradation; however above 1.0 g/L peptone was inhibitory. Similar studies showed that the addition of non-toxic compounds may stimulate the viability of cells and enhance degradation. [34] It was proposed that the presence of a more metabolisable carbon source permitted more rapid growth and the activity of the phenol degradation pathway was suppressed in order to quicken biomass acclimation to glucose as the alternate carbon source. [35]

#### **Effect of dissolved oxygen concentration**

Aerobic microorganisms utilize oxygen primarily as the terminal electron acceptor for aerobic respiration. In addition, molecular oxygen is required as a co substrate for the microbial degradation of wide variety of organic chemicals; including hydrocarbons and aromatic ring compounds. The dissolved oxygen (DO) level is the key factor which decides the rate of degradation of the organic load in aerobic growth conditions. Much of the work on the effects of dissolved oxygen concentration has been concerned with its effects on microbial growth and respiration rate. In general; bacterial respiration does not appear to be affected above a critical dissolved oxygen concentration. The critical dissolved oxygen

concentration has been defined as the concentration at which the respiration rate of the cells is one-half of the maximum rate observed at saturating levels; it is generally lower for dispersed cultures than for flocculant cultures.

#### **Phenol degradation by immobilized cells**

Phenol biodegradation has been studied in detail using pure and mixed cultures of suspended bacteria. However, at high concentrations of phenol inhibits microbial growth. [31] Several strategies have been proposed to overcome substrate inhibition. These include cell acclimation to higher concentrations of phenol, [36] the use of genetically engineered microorganisms and cell immobilization. The entrapment of biological agents in a gel matrix, [37] is quite effective, but several factors affect the specific activity of the immobilized biocatalyst when compared with free cells in on is an effective way to maintain continuous substrate degradation with concomitant cell growth for the treatment of toxic materials. In comparison with the suspension cells include the retention of microorganisms in the reactor and hence protections of cells against toxic substance are studied. [20] For the purpose of the immobilization techniques other than *Pseudomonas putida* species, various yeast are also used such as *Trichosporan* species and *Candida* species which can degrade high levels of phenol or phenolic compounds. [38]

Immobilized cells offer the possibility of degrading higher concentrations of toxic pollutants than can be achieved with free cells. It has been shown by several workers that immobilized microorganisms are better protected against phenolic compounds than are free cells. [39] The efficiency of the phenol degradation could be further enhanced by the process of cell immobilization. Alginates represent

however several advantages such as high porosity and chemical stability with a mild, fast, simple and low cost immobilization method.

## CONCLUSION

Degradation of phenol and related phenolic compounds using various microorganisms has been the topic of scientific interest for a number of decades. A large number of natural and synthetic organic compounds are biodegradable by microorganisms as part of their normal metabolism for energy and growth. A portion of the organic material, serving as a primary electron and energy source, is converted to oxidized end products through oxidation/reduction reactions. The other portion of the organic carbon is synthesized into cellular material. Such conversions can take place in aerobic environments, in which oxygen serve as the terminal electron acceptor. They also occur in anaerobic environment, in which nitrate, sulfate, carbon dioxide, other oxidized inorganic elements, or the organic compounds themselves serve as electron acceptors. Biological processes using microbial systems provide an alternative to the existing physical/ chemical technologies because they are more cost-effective, environment friendly and do not produce large quantities of sludge. Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO<sub>2</sub>, H<sub>2</sub>O, NO<sub>3</sub> and other inorganic compounds. Practical application of microorganisms for the degradation of phenol presently use almost exclusively for treatment of industrial sewage, both pure and mixed cultures of microorganisms and immobilization of cells by using various reactors opens interesting prospects for phenol degradation.

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How to cite this article: Rajani V. Microbial degradation of phenol- a review. Int J Res Rev. 2015; 2(2):46-54.

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