

## Biodegradation of Phenol: A Review

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### ABSTRACT

Phenol is one of the organic pollutants in industrial waste water which causes significant environmental problems. Various methods such as chlorination, flocculation, adsorption etc. have been used for the degradation of phenol. But biological methods have proved to be the most effective and economical approach for the removal of phenol and phenol related compounds. Numerous studies on biodegradation of phenol using different types of microorganisms and bioreactors have been reported. Various kinetic models and the effect of various parameters such as initial phenol concentration, temperature, pH, etc. on biodegradation have been also studied.

**Key words:** biodegradation, bioreactor, microorganisms, phenol

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### I. INTRODUCTION

Organic pollutants comprise a potential group of chemicals which may lead to hazardous effects on the environment. Phenol is such an organic, aromatic compound that occurs naturally in the environment, but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing etc. The waste water generated from these industrial activities contains high concentration of phenolic compounds which eventually may reach down to streams, rivers, lakes and soil, which represent a serious ecological problem. Phenol is a listed priority pollutant by the U.S Environmental protection Agency (EPA, 1979) and is considered to be a toxic compound by the Agency for Toxic Substances and Disease Registry (ATSDR, 2003). Death among adults has been reported with ingestion of phenol ranging from 1 to 32g [1]. Phenol can cause body disorders like allergic dermatitis, skin irritation, cancer and genetic disorders like mutation. It causes taste and odour problem in drinking water [2]. The increasing presence of phenols represents a significant environmental toxicity hazard, therefore the development of methods for the removal of phenols from industrial waste-water has generated significant interest. Conventional processes have been mostly physicochemical processes, but since they cause secondary problems in the effluents, biological treatments are preferred for large scale removal of this type of pollutants [3]. Currently biological methods have been widely used as a low cost alternative and offered the possibility of complete mineralization of organic compounds [4].

### II. THEORETICAL ASPECTS

#### 2.1 Structure and properties of phenol

The structure of phenol is that of a hydroxyl group (-OH) bonded to a phenyl ring. Phenol, also known as carbolic acid, is a toxic white crystalline solid with a sweet tarry odour. It is commonly referred to as a hospital smell. Its chemical formula is  $C_6H_5OH$ . The word phenol is also used to refer to any compound that contains a six-membered aromatic ring, bonded directly to a hydroxyl group (-OH). In effect, phenols are a class of organic compounds of which phenol is the simplest member. Its chemical name is hydroxybenzene.

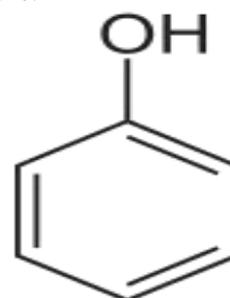


Fig1: structure of phenol

#### 2.2 Toxicity of phenol

Acute exposure of phenol causes central nervous system disorders. It leads to collapse and coma. It can also result in myocardial depression. Renal damage and salivation may be induced by continuous exposure to phenol. It is also suspected that phenol may cause paralysis, cancer and genotoxicity. Phenol is an antiseptic agent and is used in surgery, which indicates that they are also toxic to many microorganisms [5].

### 2.3 Industrial production of phenol

Phenol can be made from the partial oxidation of benzene, by the reduction of benzoic acid by the cumene process, or by the Raschig-Hooker process. It can also be found as a product of coal oxidation.

### 2.4 Uses of phenol

Phenol has antiseptic properties. It is also the active ingredient in some oral analgesics such as chloraseptic spray. It is used in the production of drugs, herbicides and synthetic resins.

### 2.5 Technologies for phenol degradation

The probable technologies for the treatment of waste water containing phenol and thiocyanate include chlorination, ozonation, adsorption, flocculation and biological treatment. The physicochemical methods have the inherent draw backs due to the tendency of formation of secondary toxic materials such as cyanates, chlorinated phenols, hydrocarbons etc. Moreover these techniques have been found to be very expensive due to large consumption of chemicals.

Important biological methods like activated sludge, trickling filter, oxidation ponds, lagoons etc have been employed for the treatment of phenolic waste water with various efficiencies depending upon the operating process variables like temperature, pH, dissolved oxygen and nutrients containing nitrogen, phosphorous and minerals[6].

## III. BIODEGRADATION OF PHENOL

### 3.1 Microorganisms and enzymes involved in phenol biodegradation

Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria, fungi and actinomycetes. Bacterial species include Bacillus sp, Pseudomonas sp, Acinetobacter sp, Achromobacter sp etc. Fungi share a significant part in the recycling of aromatic compounds in the biosphere and several studies have shown that diverse fungi are capable of phenols mineralization[7]. Fusarium sp, Streptomyces sp, Phanerocheate chrysogenum, Coriarius versicolor, Ralstonia sp etc. proved to be efficient fungal groups in phenol biodegradation[5]. V.Arutchelvan et al(2006) studied the biodegradation of phenol using the pure culture of Bacillus brevis. The bacterial strain was isolated from phenol-formaldehyde resin manufacturing industrial waste water. Harrison et al(2004) studied the biodegradation of phenol, o-cresol, m-cresol and p-cresol by indigenous soil fungi in soil contaminated with cresoate. Seven non-basidiomycetic fungi were isolated. Maria Kopytko et al (2008) studied the biodegradation of potential of phenol in industrial waste water from

an oil field. E.S.Shumkova et al(2009) studied the phenol degradation by Rhodococcus opacus 1G strain. This strain was isolated from petroleum contaminated soils. Dajun et al (2018) studied the biodegradation of phenol by Pleurotus laccase in the direct current electric field. Guruswamy Annadurai et al(2002) investigated the biodegradation potential of phenol using mixed liquors of Pseudomonas putida ATCC 31800 and activated sludge.

There are reports on many microorganisms capable of degrading phenol through the action of variety of enzymes. These enzymes may include oxygenases, hydroxylases, peroxidases, tyrosinases and oxidases. Oxygenases include monooxygenases and dioxygenases[5].

### 3.2 Mechanisms of phenol biodegradation

In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation in which the aromatic ring is initially monohydroxylated by a monooxygenase called 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 upon the type of strain, the catechol then undergoes a ring cleavage which can occur either at the ortho position thus initiating the ortho pathway that leads to the formation of succinyl Co A and acetyl CoA at the meta position, thus initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde [1]. Anaerobically the aromatic ring is not oxidized, but reduced. The key intermediate in this pathway is cyclohexanone[14].

**Table 1:** Microorganisms Involved in the Biodegradation of Phenolic Compounds

Sl No:	Type of phenol related compounds	microorganisms	Reference
1	Phenol	Pseudomonas fluorescense	[1]
2	Phenol	Bacillus brevis	[7]
3	Phenol	Pseudomonas sp	[4]
4	Phenol	Candida tropicalis	[15]
5	Phenol	Pseudomonas putida	[16]
6	Phenol	Trametes versicolor	[17]
7	Phenol & p-cresol	Scydosporium apiospermum	[18]
8	Phenol & m-cresol	Mixed microbial culture	[19]
9	Phenol & cresol	Aspergillus awamori	[20]
10	p-cresol	Aspergillus fumigatus	[21]
11	4-ethyl phenol	Aspergillus fumigatus	[22]
12	Phenol	Candida tropicalis NCTM 3556	[23]
13	Phenol & alkyl phenols	Mixed culture	[24]
14	m-cresol	Mixed microbial culture	[25]
15	Phenol	Aspergillus fumigatus	[26]

Table 2: Enzymes Involved in the Biodegradation of Phenolic Compounds

Sl No.	Type of phenol related compounds	Enzymes	Reference
1	Phenol	Phenol hydroxylase	[27]
2	Phenol	Laccase	[28]
3	Phenol	Laccase, polyphenol oxidase	[17]
4	p-Cresol	Methyl hydroxylase	[21]
5	Phenol	Phenol hydroxylase, Catechol, 2-dioxygenase	[29]
6	Phenol	Laccase	[30]
7	4-ethyl phenol	Methylene hydroxylase	[22]
8	Phenol	Phenol hydroxylase	[31]
9	Phenol	Catechol, 2-dioxygenase, Catechol, 3-dioxygenase	[11]
10	Phenol	Laccase, polyphenol oxidase	[17]

### 3.3 Effect of various factors on biodegradation of phenol

There are various factors which affect the biodegradation of phenolic compounds. V.Arutchelvan et al (2006) studied the effect of initial phenol concentration, temperature, pH, inoculum size, addition of co-substrate were studied. Biodegradation of phenol was maximum at pH 8.0, 5%v/v of inoculum size and without any co-substrate. Mailin Mission & Firdausi Razali(2007) investigated the performance of two types of inert supports namely bioceramic and sponge to immobilize a locally isolated phenol degrader *Pseudomonas* sp in a packed column in repeated batch culture. Complete phenol removal was obtained between 250-1000ppm.

### 3.4 Bioreactors used in phenol biodegradation

G.Venkat Reddy et al(2006) carried out mass transfer studies on biodegradation of phenolic waste water in draft tube fluidized bed reactor. The mass transfer coefficient for transport of phenol from the bulk phase to the solid particle has been measured experimentally. The mass transfer coefficient for phenol was varied between 0.06 and  $0.24 \times 10^5$  m/s. Pichiah Saravanan et al(2008) carried out a comparative study on the performance of batch stirred tank bioreactor and internal air lift bioreactor in degrading phenol using *Pseudomonas* spp. BSTR took a maximum of 8 days to degrade phenol completely with maximum initial concentration of 400 mg l<sup>-1</sup>; whereas ILALR took only 47 h to degrade phenol with a maximum initial concentration of 66 mg l<sup>-1</sup>. I. Alemzadeh et al (2002) carried out phenol biodegradation by rotating biological contactor. The impact of major process and operating variables such as input hydraulic loading, input COD loading and temperature of waste water on the total removal efficiency of the system were examined.

### 3.5 Kinetics of phenol biodegradation

A variety of kinetic substrate utilization and inhibition models have been used to describe

the dynamics of microbial growth on phenol. Of these various models, the Monod and Andrew (Haldane) equations has been extensively used to describe phenol biodegradation. The Monod and Haldane equations are based on the specific growth rate, but may also be related to the specific substrate consumption rate [1]. S.E. Agarry et al (2007) estimated kinetic parameters under substrate limitation using mixed culture containing *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* isolated from an oil polluted area in Niger-Delta regions of Nigeria. It fit best for Teisser kinetic inhibition model. M.Rigo and R.M. Alegria(2004) determined the kinetic parameters of phenol biodegradation in a batch reactor. *Candida tropicalis* was found to be capable of growth on a medium with 1g/l phenol. Haldane equation was found to be fit to the experimental data. V. Vijayagopal and T. Viruthagiri(2005) aimed to determine the kinetics of phenol biodegradation by measuring the biomass growth rate and phenol concentration as a function of time in a batch reactor. Kinetic constants were determined using Haldane equation.

Sl No.	Author/Year	Microorganism	Reactor	Parameters studied	Model used
1	K. Bandyopadhyay et al 2001	<i>Pseudomonas putida</i> MTCC 1194	Batch	pH, temperature, initial substrate concentration, particle size, cell loading	Michaelis-Menten kinetics
2	Kuo-Ling Ho et al 2009	<i>Corynebacterium</i> sp.D11	Batch	Inoculum concentration, pH, temperature	Haldane
3	Ivanka Stobova et al 2006	<i>Aspergillus oryzae</i> NRRL 5112	Batch	Inoculation time	Haldane
4	Arijay Kumar et al 2005	<i>Pseudomonas putida</i> MTCC 1194	Batch	Initial phenol concentration	Haldane
5	G.Venkat Reddy et al 2006	<i>Pseudomonas putida</i> NCIM 2176	Draft tube fluidized bed	Mass transfer coefficient	Monod

Table 3: Biodegradation of phenol

## IV. CONCLUSION

Biodegradation of phenolic compounds by microorganisms is an efficient, economically feasible and environmental friendly technology. A variety of microorganisms have proven their ability to degrade phenol and phenol related compounds. Several models have been proposed to study the kinetics of phenol biodegradation. The Monod and Haldane model are the most commonly used kinetic models.

## REFERENCES

- [1]. S.E. Agarry, B.O. Solomon, Kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescens*, International Journal of Environmental Science Technology 5,2,2008,223- 232.
- [2]. Gurusamy Annadurai, Lai Yi Ling, Jiunn-Fwu Lee, Statistical optimization of medium

- components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*, *Journal of Hazardous Materials*, 151,2008,171-178
- [3]. B. Marrot, A. Barrios – Martinez, P. Moulin, N. Roche, Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor, *Biochemical Engineering Journal*, 30 ,2006, 174 - 183.
- [4]. Mailin Misson & Firdausi Razali, Immobilization of phenol degrader *Pseudomonas sp* in repeated batch culture using bioceramic and sponge as support materials, *Journal of Technology* ,46 ,F ,2007,51 - 59.
- [5]. .C.Indu Nair,K.Jayachandran, ShankarShashidar, Biodegradation of Phenol, *African Journal of Biotechnology*,7,25,2008,4951-4958.
- [6]. K.Bandhyo Padhyay, D.Das, P.Bhattacharyya, B.R.Maiti, Reaction engineering studies on biodegradation of phenol by *Pseudomonas putida* MTCC 1194 immobilized on calcium alginate, *Biochemical Engineering Journal* 8,2001, 179 - 186.
- [7]. Taghreed Al-Khalid and Muftah.H.El-Naas,Aerobic Biodegradation of Phenols:A Comprehensive
- [8]. V.Arutchelvan, V.Kanakasabai, R.Elangovan, S.Nagarajan, V.Muralikrishnan, Kinetics of high strength phenol degradation using *Bacillus brevis*, *Journal of Hazardous Materials B129*, 2006, 216 – 222
- [9]. Harrison Ifeanyichukwu Atagana, Biodegradation of phenol, o-cresol, m-cresol and p-cresol by indigenous soil fungi in soil contaminated with creosote, *World Journal of Microbiology & Biotechnology* 20, 2004, 851-858
- [10]. Maria Kopytko, Luz Adriana Puentes Jacome, Alternative for Phenol Biodegradation of Contaminated waste waters using an adapted bacterial biofilm layer , *Rudarsko-geolosko-naftni zbornik* ,20, 2008, 71-82
- [11]. E.S.Shumkova, I.P.Solyanikova, E.G.Plotnikova, and L.A. Golovleva, Phenol degradation by *Rhodococcus opacus* strains 1G, *Applied Biochemistry and Microbiology*,45 ,1,2009, 43 - 49.
- [12]. Dajun Ren, Xiaoyong He, Chunxing Wang, Shuqing Zhang, Biodegradation of phenol by *Pleurotus laccase* in the direct current electric field,*Biomedical Research* 2018, Special Issue: S349-S351
- [13]. Gurusamy Annadurai,Ruey-Shin Junag, Duu-Jong Lee, Microbiological degradation of phenol using mixed liquors of *Pseudomonas putida* and cultivated sludge, *Waste Management* 22,2002, 703-710
- [14]. M.Rigo,R.M.Alegre, Isolation and selection of phenol degrading microorganisms from Industrial waste water and kinetics of the biodegradation, *Folia Microbiol*,49,1,2004,41-45
- [15]. Nora Ruiz - Ordaz, Juan carlos Ruiz - Langunez, Jose Humberto castanon - Gonzalez, Eli zabeth Hernandez - Manzano, Eliseo Cristiani -Urbina, and Juvencio Galindez - mayer, Phenol biodegradation using a repeated batch culture of *Candida tropicalis* in a multistage bubble colum, *Revista Latinoamericana de Microbiologia* 43 ,2001,19 -25.
- [16]. Zumriye Aksu and Gultae Bulbul, Investigation of the combined effects of external mass transfer and biodegradation rates on phenol removal using immobilized *P.putida* in a packed bed column reactor, *Enzyme and microbial Technology*,22,1998,397-403
- [17]. C.Udayasoorian and P.C.Prabhu, Biodegradation of phenols by lignolytic fungus *Trametes versicolor*, *Journal of Biological Science*, 5,6,2005,824-827
- [18]. M. caluben and S.Schmidt, Biodegradation of Phenol and p-cresol by the hyphomycete *Scedosporium apiospermum*, *Res.Microbiol* 149,1998,39-46
- [19]. Pichiah Saravanan, K. Pakshirajan, Prabirkumar Saha , kinetics of phenol and m-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant, *Journal of Environmental Sciences* 20,2008, 1508-1513
- [20]. Hyusein Yemendzhiev, Maria Geviginova, Plamena Zlateva, Ivanka Stoilova, Albert Krastanov and Zlatka Alexieva,Phenol and cresol mixture degradation by *Aspergillus awamori* strain: Biochemical and kinetic substrate interactions, *Proceedings of ECOpole 2*,1,2008,153-159
- [21]. Kerina. H. Jones, Peter W Trudgill and David. J Hopper, metabolism of p-cresol by the fungus *Aspergillus fumigatus*, *Applied and Environmental Microbiology* 59,4 ,1993, 1125 - 1130.
- [22]. Kerina. H. Jones, Peter W Trudgill and David. J Hopper, 4-Ethyl phenol metabolism by *Aspergillus fumigatus*, *Applied and*

- Environmental Microbiology 60,6 ,1994, 1978 - 1983.
- [23]. Rita.J.Varma, Bhaskar.G.Gaikwad, Rapid and high biodegradation of phenols catalyzed by *Candida tropicalis* NCIM 3556 cells, *Enzyme and Microbial Technology* 43,2008,431-435.
- [24]. M.E.Acuna-Arguelles, P-olgium-Lora of Razo-Flores, Toxicity and kinetic parameters of the aerobic biodegradation of phenol and alkyl phenols by a mixed culture, *Biotechnology Letters* 25,2003, 559-564
- [25]. Pichiah Saravanan, K. Pakshirajan, Prabirkumar Saha, Batch growth kinetics of an indigenous mixed microbial culture utilizing m - cresol as the sole carbon source; *Journal of Hazardous Materials* 162 ,2009, 476 – 481
- [26]. P.Balamurugan , B.Preetha and T.Virithagiri, Study on effect of operating parameters on biodegradation of phenol by *Aspergillus fumigatus*, *International Journal of Engineering Research and Applications* , 2, 2,2012, 981-986
- [27]. Christian Muller,Lothar Petruschka, Heinrich Cuypers, Gerhard Burchardt and Heidrun Herrmann, Carbon catabolite repression of phenol degradation in *Pseudomonas putida* is mediated by the inhibition of the activator protein PhIR, *Journal of Bacteriology* 178,7,1996,2030-2036
- [28]. Cordi.L, Minussi.R.C Freire.R.S Duran.N, Fungal laccase: copper induction, semipurification, immobilization, phenolic effluent treatment and electro chemical measurement, *African Journal of Biotechnology* 6,10,2007,1255-1259
- [29]. V.L.Santos, Valter.R.Linardi, Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation of phenol, *Process Biochemistry*39, 2004, 1001-1006
- [30]. A.Lante,A.Crapisi,A.Krastanov,P.Spettoli, Biodegradation of phenols by laccase immobilized in a membrane reactor, *Process Biochemistry*,36, 2000,51-58
- [31]. Moktar Hamdi, Abdelkader Khadir and Jean-Louis Garcia, The use of *Aspergillus niger* for the bioconversion of olive mill waste waters, *Applied Microbiology and Biotechnology* 34, 1991, 828-831.
- [32]. G.Venkat Reddy,A.Venuvinod and Ch.Ravi Shankar,Mass transfer studies on biodegradation of phenolic waste water in draft tube fluidized bed bioreactor, *Indian Chemical Engr.Section A*, 48,2,2006.
- [33]. Pichiah Saravanan, K. Pakshirajan, Prabirkumar Saha, Performance of batch stirred tank bioreactor and internal loop airlift bioreactors in degrading phenol using *Pseudomonas* spp:- A competitive study, *Journal of Environmental Protection Science* 2 ,2008,.81-86
- [34]. I.Alemzadeh, F.Vossoughi,M.Houshmandi,Phenol biodegradation by rotating biological contactor, *Biochemical Engineering Journal* 11,2002, 19-23
- [35]. S.E.Agarrry, E.Betiku, B.O.Solomon, Inhibition kinetics of phenol Degradation of binary mixed culture from continuous culture and wash-out data,*Journal of Engineering and Applied Sciences* 2,6,2007,1020-1026.
- [36]. V.Vijayagopal and T.Viruthagiri,, Batch kinetic studies in phenol biodegradation and comparison, *Indian Journal of Biotechnology* 4,2005, 565-567
- [37]. Kuo - Ling Ho, Bin Lin, Yu-You chen, Duu-Jong Lee, Biodegradation of phenol using *Corynebacterium* sp DJI aerobic granules, *Bio resource Technology* 100, 2009,5051 - 5055.
- [38]. Ivanka Stoilova, Albert Krastanov, Veselin Stanchev, David Daniel,Maria Gerginova, Zlatka Alexieva, Biodegradation of high amounts of phenol,Catechol, 2,4-dichlorophenol and 2,6-dimethoxy phenol by *Aspergillus awamori* Cells,*Enzyme and Microbial Technology* 39,2006, 1036-1041
- [39]. Arinjay Kumar,Shashi Kumar, Surrendered Kumar, Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194, *Biochemical Engineering Journal* 22, 2005, 151-159
- [40]. Pichiah Saravanan,Kannan Pakshiraja, Prabirkumar Saha, Performance of batch stirred tank bioreactor and internal loop airlift bioreactors in degrading phenol using *Pseudomonas* spp:-A comparative study, *Journal of Environmental Protection Science*,2,2008,81-86.

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