



REVIEW ARTICLE

Recent advances in the Biodegradation of Phenol: A review

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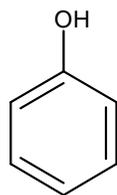
ABSTRACT

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. 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, and various reactors used for biodegradation.

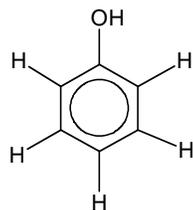
Key words: Phenol, biodegradation, microorganisms, reactors

INTRODUCTION

Phenol is one of the most widely used in the organic compounds in existence and is a basic structural unit for a variety of synthetic organic compounds including agricultural chemicals and pesticides. Phenol is naturally found in decaying dead organic matters like rotting vegetables and in coal. The German chemist, Runge isolated phenol from coal tar in 1834 and named karbolsaure (coal-oil acid or carboic acid), though its composition was not known until 1841. Phenol (hydroxy benzene) (Figure 1) is both a synthetically and naturally produced aromatic compound. It was first used in the raw state, as creosote, to prevent the weathering of railway ties and ships timber, and to reduce the odour of decomposition in sewage. At room temperature phenol is a translucent, colorless, crystalline mass, white powder or syrupy liquid when mixed with water. The crystals are hygroscopic and turn pink to red in air. Phenol has a sweet tar like odour and is soluble in alcohol, glycerol, petroleum and water to a lesser extent.



Phenol



Hydroxybenzene

Figure 1. Chemical structures of phenol

Naturally derived phenol is obtained by fractional distillation of coal tar, mostly phenol is made from 1-methylethylbenzene (cumene), which can be used as an indication of the levels of phenol production. The world annual production of 1-methylethylbenzene is shown in (Figure 2) Phenol can also be made by synthetic processes such as oxidation of toluene, fusion of sodium benzenesulfonate with sodium hydroxide or heating monochlorobenzene with sodium hydroxide under high pressure.

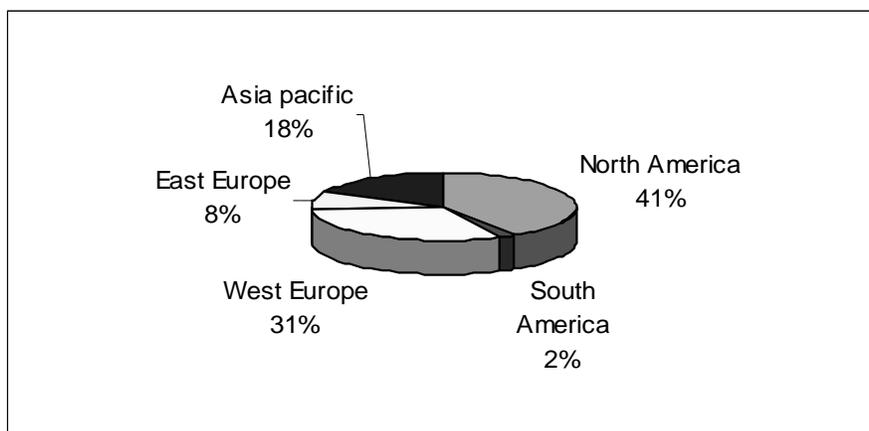


Figure 2. Annual Production of 1-methylethylbenzene - An indication of phenol production

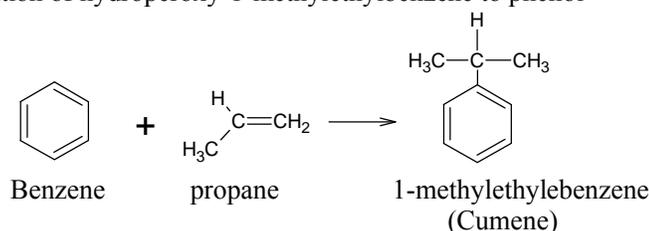
Methods of commercial phenol production:

Production by cumene route

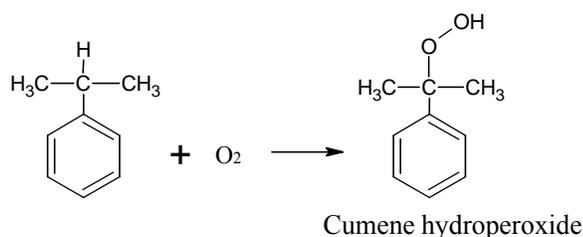
The main method used to manufacture phenol since the 1960s has been through the oxidation of 1-methylethylbenzene. The oxidation of 1-methylethylbenzene takes place in three stages,

1. Producing 1-methylethylbenzene (cumene) from benzene and propane
2. Oxidation of 1-methylethylbenzene to hydroperoxy-1-methylethylbenzene
3. Decomposition of hydroperoxy-1-methylethylbenzene to phenol

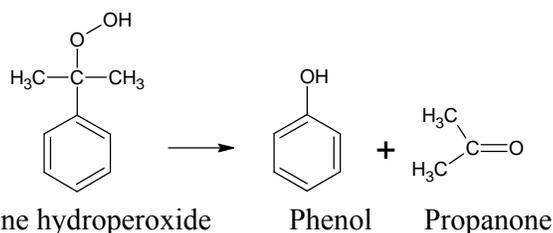
Stage-I



Stage-II



Stage-III



Phenol production through micro reactor technology

In future, many chemicals including phenol may be produced in relatively small reactors (Figure 3) about the size of a large desktop. One potential micro-reactor to produce phenol involves the use of a small diameter (2 mm), porous tube of alumina coated with a layer of palladium metal. A mixture of benzene and oxygen is fed through the tube and hydrogen gas is passed over the tube, the tube is heated to 150 - 250°C.

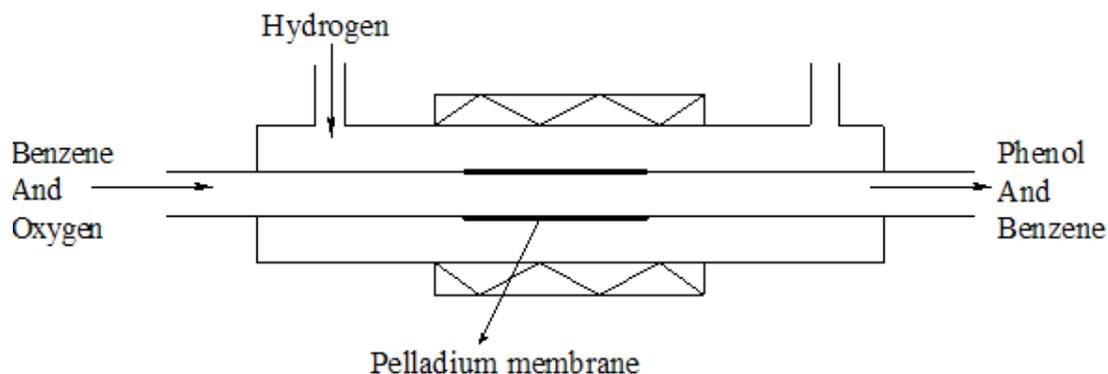
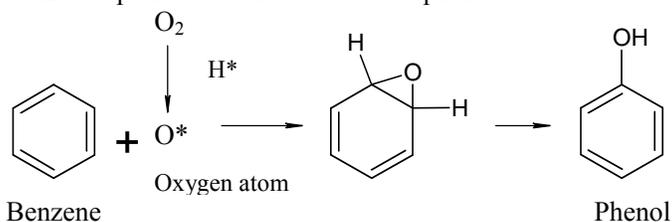


Figure 3. Schematic diagram of the Micro reactor technology

Hydrogen permeates through the alumina tube and is converted to atomic hydrogen by the palladium catalyst. The hydrogen atoms react with oxygen gas, releasing oxygen atoms, which in turn react with the benzene forming benzene epoxide which isomerises to phenol.



The boiling points of phenol (182°C) and benzene (80°C) favours the easy separation of phenol from unreacted benzene, the final liquid phenol is in a highly pure form. Researchers claim that this method saves on capital cost, reduces the energy, reduces waste and can easily be scaled up by adding more tubes effectively using a modular approach. One single micro-reactor could produce up to 100,000 tonnes per year and this technology can also be applied to the manufacture of other materials. Since phenol is a toxic and regulated chemical, its *In situ* addition to groundwater as a cosubstrate requires close monitoring of its behavior and performance. Phenol creates odour and taste problems in water at concentration of 100-1000 µg/L. Also, phenol reacts during chlorination of water to form chlorophenols, which have extremely low taste and odor thresholds below 1 µg/L and are suspected carcinogens. Therefore, phenol and oxygen delivery schedules that provide conditions that achieve complete degradation of all added phenol are necessary. In both the above-mentioned *In situ* pilot plant studies, efficient removal of phenol was demonstrated [1]. The effect of phenol when present in the waste water at the toxic level leads to carcinogenic problems. Several papers described on the degradation which includes microbial degradation, adsorption on to different matrices, chemical oxidation, solvent extraction and irradiation. The biodegradation of phenol have also been reported by using micro algae [2, 3].

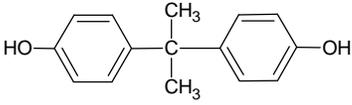
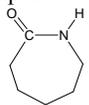
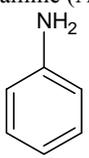
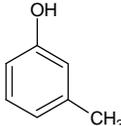
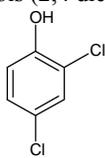
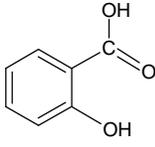
Irrespective of using various microbial techniques, the old and cost effective process that is by means of utilizing the activated carbon is also discussed in several papers. The biological removal of adsorbents on the carbon surface can reopen the adsorption sets, which can be occupied by other organic molecules in the bulk solution. Other than the activated carbon there are many adsorbents which are obtained in the nature. Some sorbents such as clay, oxide and bentonite among these sorbents bentonite has been widely used as a sorbent in earthen lines since it can strongly adsorb heavy metals via ion exchange [4].

Uses of phenol

Phenol has been in production since 1860s. Towards the end of the 19th century, industrial scientists have found many applications of phenol in the synthesis of dyes, aspirin, and one of the first high explosives, picric acid. As early as in 1872, it was found that phenol could be condensed with aldehydes (for example methanal) to make resinous compounds, a process still in use today. Phenol-methanal (formaldehyde) resins are the basis of the oldest plastics, still used to make low cost thermosetting plastics such as melamine and bakelite used in electrical equipment. These resins are also used extensively as bonding agents in manufacturing wood products such as plywood. Phenol is

also used to make chemical intermediates for a wide range of other applications ranging from plastics to pharmaceuticals and agricultural chemicals (Table 1).

Table 1. Chemical intermediates

Intermediate	End use/ Product
<p>Bisphenol A</p> 	Used to produce epoxy resins for paints coatings and mouldings, and in polycarbonate plastics, familiar in CDs and domestic electrical appliances
<p>Caprolactam</p> 	Caprolactam is used in the manufacture of nylon and polyamide plastics for a wide range of products, including carpets, clothing, fishing nets, moulded components and packaging
<p>Phenylamine (Aniline)</p> 	This is used as an antioxidant in rubber manufacture, and as an intermediate in herbicides, dyes and pigments, and pharmaceuticals. It is used to make isocyanates for the production of polyurethanes, with a wide range of uses from paints and adhesives to expanded foam cushions
<p>Alkylphenols (3-methylphenol)</p> 	These compounds are used in the manufacture of surfactants, detergents and emulsifiers, and also in insecticide and plastics production
<p>Chloro-phenols (2,4 dichlorophenol)</p> 	Used in medical antiseptics and bactericides such as TCP and Dettol. Also used in fungicides for timber preservation and as additives to inhibit microbial growth in many products; used to manufacture a range of pesticides
<p>Salicylic acid</p> 	This is used in the production of aspirin and other pharmaceuticals

Removal of phenol by solvent extraction, adsorption, chemical oxidation, incineration and other non biological methods have serious drawbacks such as high cost and formation of hazardous byproducts. Where as biological degradation is generally preferred due to lower costs and possibility of complete mineralization. The significance of this study is to describe the production of phenol, degradation of phenol in aerobic and anaerobic pathways, various types of reactors used for the phenol degradation, kinetic study of the microbial degradation of phenol by various microorganisms in the form of pure, mixed culture and immobilized cells, the parameters like temperature, pH, DO and the effect of additional carbon source that influence the degradation of phenol and the health effects were discussed. This study could contribute to a better understanding of the ability of the immobilized microorganisms to handle high strength industrial wastewaters containing chemicals that are inhibitory to microbial growth and biodegradation.

DEGRADATION OF PHENOL THROUGH AEROBIC AND ANAEROBIC PATHWAYS

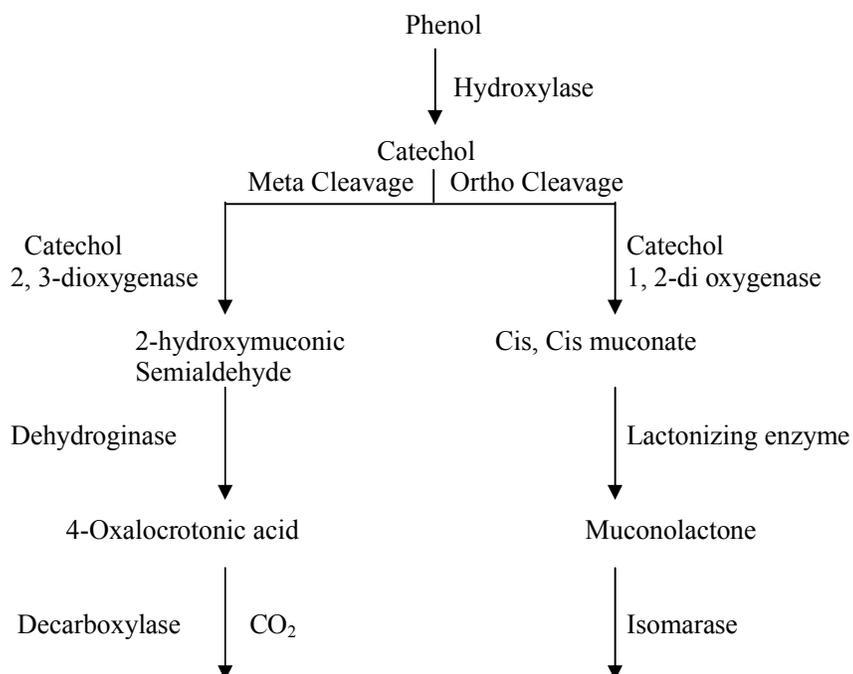
Because of widespread occurrence of phenol in the environment many microorganisms utilize phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms.

Aerobic biodegradation of phenol

Aerobic biodegradation has been studied in the early 19th centuries. In the first step of the aerobic pathway (Figure 4) for the biodegradation of phenol, molecular oxygen is used by the enzyme phenol hydroxylase to add a second hydroxyl group in ortho-position to the one already present in which reaction requires a reduced pyridine nucleotide (NADH₂). The resulting catechol (1, 2-dihydroxybenzene) molecule can then be degraded via two alternative pathways depending on the responsible microorganism. In the ortho- or β -keto adipate pathway, the aromatic ring is cleaved between the catechol hydroxyls by a catechol 1, 2-dioxygenase (intradiol fission) [5, 6]. Preliminary evidence for the production of β -keto adipate during the degradation of phenol by strain '*Vibrio OI*' was first presented by Evans and Kilby [7, 8]. The resulting cis, cis muconate is further metabolized via β -keto adipate to Krebs cycle intermediates. In the meta-pathway, ring fission occurs adjacent to the two hydroxyl groups of catechol (extradiol fission). The enzyme catechol 2, 3-dioxygenase transforms catechol to 2-hydroxymuconic semialdehyde. This compound is metabolized further to intermediates of the Krebs cycle. The organisms which utilize phenol by aerobic pathway are *Acinetobacter calcoeticus*, *Pseudomonas* species and *Candida tropicalis* and most of the eukaryotes typically employ ortho pathway. The aerobic genus *Pseudomonas* species have been subject to various studies and its versatility to utilize a wide spread of aromatic substrates makes it an attractive organism for use in waste water treatment applications.

Anaerobic biodegradation of phenol

Phenol can also be degraded in the absence of oxygen and it is less advanced than the aerobic process. It is based on the analogy with the anaerobic benzoate pathway proposed for *Paracoccus denitrificans* in 1970 [9]. In this pathway phenol is carboxylated in the para position to 4-hydroxybenzoate which is the first step in the anaerobic pathway. Here the enzyme involved is the 4-hydroxy benzoate 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 methogenic consortium was later shown to travel a variety of phenolic compounds including o-cresol, catechol and ortho halogenated phenols via para carboxylation followed by dehydroxylation. The organisms capable of degrading phenol under anaerobic conditions were *Thauera aromatica* and *Desulphobacterium phenolicum*.



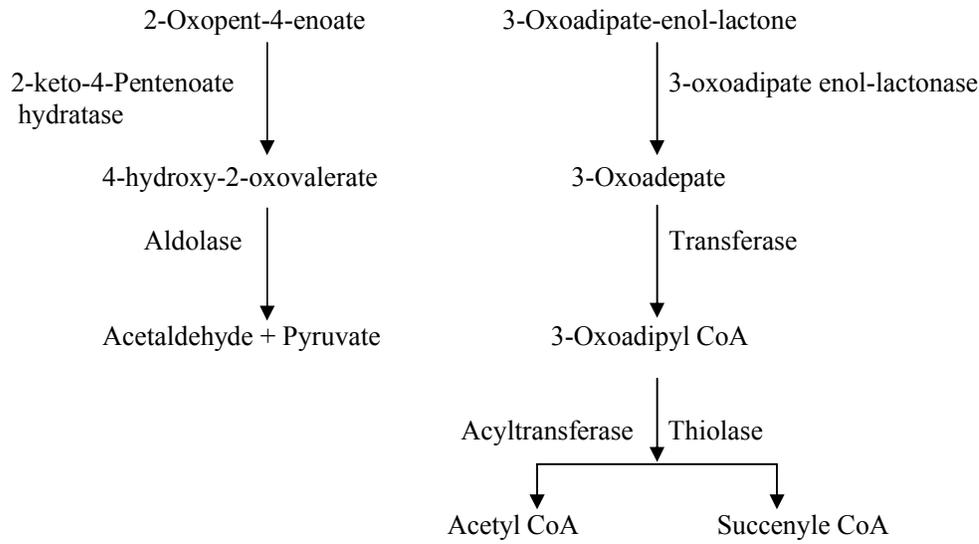
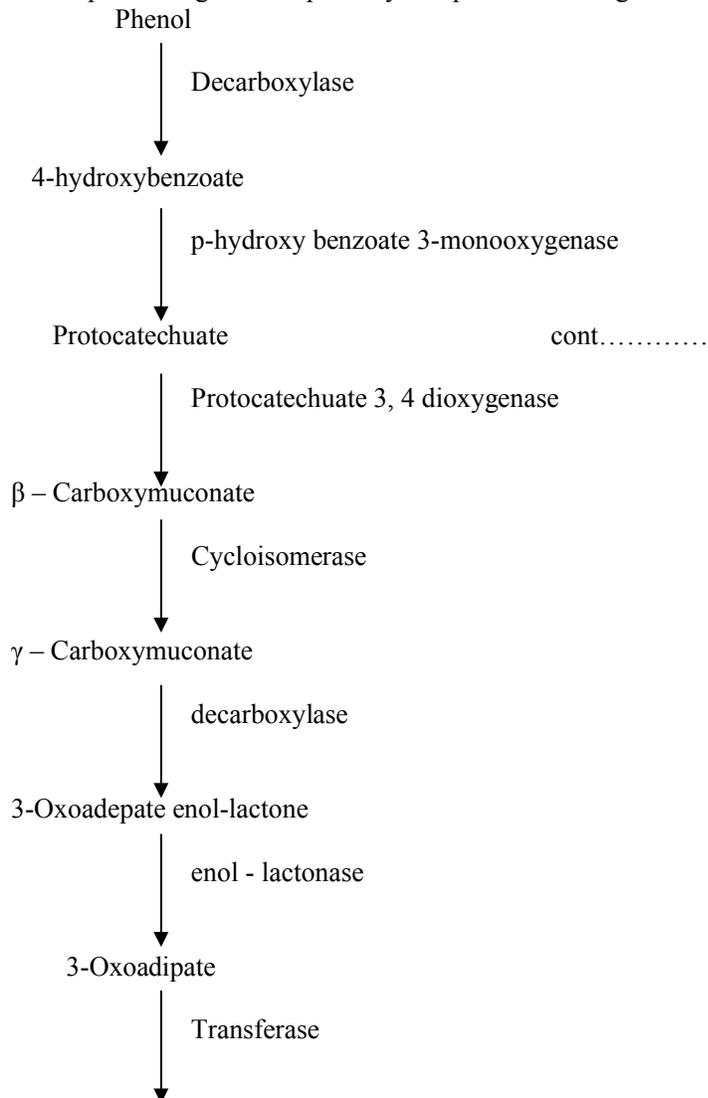


Figure 4. Flow chart of aerobic degradation pathway for phenol

A schematic overview of the anaerobic phenol degradation pathway is represented in Figure 5



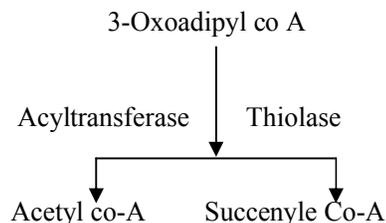


Figure 5. Flow chart of anaerobic degradation pathway for phenol

VARIOUS MICROORGANISMS INVOLVED IN PHENOL DEGRADATION

Phenol, an aromatic hydrocarbon is degraded by various microorganisms, (Table 2) 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 [10]. It has been reported that the *Pseudomonas* species follows a typical meta cleavage pathway for metabolizing phenol at relatively low concentrations [11].

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 [12]. *Trichosporon cutaneum*, *candida* species, *Rhodotorula* species were able to utilize phenol as sole source of carbon and energy [13] *Fusarium flocciferum* [14] white rot fungi [15] *Phanerochaete chrysosporium* [16, 17] 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. Recent studies indicate that the 4-chlorophenol, 4-nitrophenol and phenol cause adaptive effects in the membrane of *Aspergillus chlorophenicus*. Degradation of monochlorophenols as sole source of carbon in aerobic batch culture has been examined by mixed microbial community [18]. 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 [19]. 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.

VARIOUS REACTORS FOR PHENOL DEGRADATION

The presence of phenolic compounds in water and soil has become significant problems. Common commercial wastewater treatment methods utilize the combination of physico-chemical and biological treatment. Both chemical and biological processes were used for many years to treat phenolic wastewater. Activated sludge, fluidized, packed-bed and moving bed biofilm reactors were studied as biological treatment processes. Table 3 shows various reactors used in phenol degradation and their effect. Phenol degradation using an anaerobic packed-bed reactor was reported by Holladay *et al.* [49] and also compared phenol degradation in stirred tank, packed bed and fluidized bed reactors. It was concluded that the efficiency for degrading phenolic liquid among the three types of bioreactors increased in the following order: stirred tank bioreactor, packed bed fluidized-bed bioreactor. The degradation rate depends on the state of biomass development, feed concentration, liquid flow rate, and air flow rate.

Table 2. List of various microorganisms involve in the phenol degradation

Source	Genus	Species	Reference	
Bacteria	<i>Alcaligenes</i>	<i>Alcaligenes faecalis</i>	Yan Jiang <i>et al.</i> , [20]	
		<i>Alcaligenes xylosoxidans</i> Y234	Sung Ho Yeom <i>et al.</i> , [21]	
	<i>Arthrobacter</i>	<i>Arthrobacter species</i>	S.Kar <i>et al.</i> , [22]	
		<i>Arthrobacter citreus</i>	Chandrakant <i>et al.</i> , [23]	
	<i>Pseudomonas</i>	<i>Arthrobacter chlorphenolicus</i> A6	Maria Unell <i>et al.</i> , [24]	
		<i>Pseudomonas putida</i>	Annadurai <i>et al.</i> , [25] Bandyopadhyay <i>et al.</i> , [26]	
		<i>Pseudomonas cepacia</i>	Folsam <i>et al.</i> , [27]	
		<i>Pseudomonas pictorum</i>	Annadurai <i>et al.</i> , [28]	
		<i>Pseudomonas aeruginosa</i> MTCC 4996	Kotresha <i>et al.</i> , [29]	
		<i>Pseudomonas aeruginosa</i>	Ahmed, [30]	
		<i>Pseudomonas aeruginosa</i> CC7CCAB919095		
		<i>Cyanobacterium synechococcus</i>	Song <i>et al</i> [31]	
		<i>Bacillus species strain PHN 1</i>	Wurster <i>et al.</i> , [32]	
		<i>Bacillus brevis</i>	Tallur <i>et al.</i> , [33]	
	Fungi	<i>candida</i>	<i>Candida tropicalis</i>	Sunil S <i>et al.</i> , [35], Yan Jiang <i>et al.</i> , [36]
<i>Candida tropicalis</i> NICM 3556			Varma <i>et al</i> [37, 38]	
<i>Fusarium</i>		<i>Fusarium species</i>	Weijian Cai <i>et al.</i> , [39]	
<i>Graphium</i>		<i>Graphium sp.FIB4</i>	Vera L.Santos <i>et al.</i> , [40]	
<i>Ochromonas</i>		<i>Ochromonas danica</i>	Kirk T. Semple <i>et al.</i> , [41]	
<i>Aspergillus</i>		<i>Aspergillus awamori</i> NRRL 3112	Stoilova <i>et al.</i> , [42]	
Yeast		<i>Phanerochaete</i>	<i>Phanerochaete chrysosporium</i>	Ahmadi <i>et al.</i> , [43]
		<i>Rhodococcus</i>	<i>Rhodococcus erythropolis</i> UPV-1	Begona <i>et al.</i> , [44]
	<i>Rhodotorula</i>	<i>Rhodotorula creatinivora</i>	Irina Krallish <i>et al.</i> , [45]	
	<i>Sphigmonas</i>	<i>Sphigmonas chlorphenolica</i> RA 2	Bielefeldt <i>et al.</i> , [46]	
	<i>Trichosporon</i>	<i>Trichosporon species</i> LE3	Vera L.Santos <i>et al.</i> , [47]	
		<i>Trichosporon cutaneum</i> R57	Aleksieva <i>et al.</i> , [48]	

Table 3. Reactors used in the phenol degradation

S.No	Reactor	Organisms used	Effect on phenol degradation	Reference
1	Packed bed Reactor	<i>Rhodococcus erythropolis</i>	Able to degrade completely phenol in defined mineral medium at a maximum rate of 18kg of phenol m ⁻³ per day	Begona Prieto <i>et al.</i> , 2002. [44]
2	Air stirred Reactor	<i>Rhodococcus erythropolis</i> UPV-1	Completely degrade phenol in synthetic wastewater at a volumetric productivity of 11.5 kg of phenol/m ³ /day	Begona Prieto <i>et al.</i> , 2002. [44]
3	Packed bed Reactor	<i>Alcaligenes xylosoxidans</i> Y234	Able to degrade phenol of 1000 ppm completely in 60 h	Sung Ho Yeom <i>et al.</i> , [50]
4	Hallow Fiber Membrane bioreactor	<i>Pseudomonas putida</i>	Able to degrade phenol of 1000 – 2000 mg/L	Yi Li <i>et al.</i> , [51]
5	Rotating biological contactors (RBC)	Mixed culture	Input loading 1754 – 3508 mg phenol/m ³ h	Sameer H israni <i>et al.</i> , [52]
6	Air lift bioreactor	<i>Alcaligenes xylosoxidans</i> and <i>Xanthomonas maltophilia</i>	The fractional conversion of phenol over 99% was achieved	Vladimer Bales <i>et al.</i> , [11]
7	Loop airlift bioreactor with a packed bed.	<i>Pseudomonas putida</i> ATCC 17484	100% phenol removal was achieved at phenol loading rates up to 33120 mg /h m	Hossein Nikakhtari <i>et al.</i> , [53]
8	Pulsed plate bioreactor	Immobilized <i>Nocardia hydrocarbonoxydans</i>	100% degradation could be achieved with 300 and 500ppm influent phenol concentrations and at very low dilution rate of 0.4094 1/h	Vidya Shetty <i>et al.</i> , [54]
9	Self cycling Fermentation in a stirred tank reactor	<i>Pseudomonas putida</i>	Substrate utilization rates as high as 14.5 kg of phenol per cubic meter of fermentor volume per day of fermentation	Hughes <i>et al.</i> , [55]
10	Granular activated carbon was incorporated into hollow fiber membrane bioreactor	<i>Pseudomonas putida</i>	1000 ppm phenol was removed within 25 h	Chao Wang <i>et al.</i> , [56]

KINETICS OF BACTERIAL 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. Microbial growth by binary fission is describe by (equation 1 and 2)

$$r_x = \frac{dC_x}{dt} = \mu C_x \quad (1)$$

$$\mu = \mu_{\max} \frac{C_s}{K_s + C_s} \quad (2)$$

Where μ = specific growth rate, μ_{\max} = maximum specific growth rate (1/h), C_s = substrate concentration (mg/L), K_s = substrate saturation constant (i.e. substrate concentration at half μ_{\max}) (mg/L). “ μ ” asymptotically approaches an upper limit μ_{\max} as C_s increases but substrate inhibition implies that μ must eventually decline as the substrate concentrations increases.

For the modeling bacterial growth kinetics on phenol with inhibition effect, the Haldane equation is extensively used [57] (equation 3)

$$\mu = \mu_{\max} \frac{C_s}{K_s + C_s + \frac{C_s^2}{K_I}} \quad (3)$$

Where K_I = inhibition constant (mg/L) K_s = monad constant (mg/L).

The uptake rate of substrate by microorganisms is generally related to growth. Thus the relation concerning this fact is (equation 4)

$$r_s = - \frac{r_x}{Y_{x/s}} \quad (4)$$

Where $Y_{x/s}$ is the yield coefficient which relates cell mass to substrate utilization, r_x = growth rate of cell

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 [58]. 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 [59, 60, 61]. The optimum pH for phenol degradation is 7.0 for *Pseudomonas putida* NICM 2174 [62].

Effect of temperature

Temperature plays an important role than nutrient availability in the degradation of organic pollutants. According to Pakula *et al.* [63] phenol biodegradation was significantly inhibited at 30°C. However, most laboratory studies on phenol degradation have been carried out at an optimum temperature of 30°C [64, 65]. Annadurai *et al.* [61] and Chitra [60] 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.

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. 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.

Temperature models for kinetic parameters

There are essentially two types of treatment models currently in use for Monod maximum specific growth rate (1/h). Arrhenius type models express the dependent variable as ln rate, whereas with square-root models it is expressed as rate^{0.5}.

The Arrhenius (1889) model given as:

$$\mu_m = Ae^{-\frac{\Delta H^*}{RT}} \quad (5)$$

Where μ_m = monod maximum specific growth rate (1/h), ΔH^* - Arrhenius temperature characteristic (kJ/mol).

Which includes the temperature characteristic ΔH^* which is assumed to be constant, however ΔH^* can vary as much as threefold or fourfold for microbial cultures, depending on the temperature range chosen [66].

Another approach was proposed by Ratkowsky *et al* [66] who developed an empirical square-root model as:

$$\sqrt{\mu_m} = b_1(T - T_0) \quad (6)$$

Where T = temperature (°C), T_0 = characteristic temperature in square-root model for monod maximum specific growth rate (1/h), b_1 = proportionality constant in square-root model (1/°C h^{0.5})

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 [67, 68] in which, phenol is degraded via the meta-pathway [69]. However it has been found that these bacteria suffer from substrate inhibition, whereby growth and consequently phenol degradation is inhibited at high phenol concentrations [57, 68]. 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 [70], immobilization of the cells [71] and using genetically engineered microorganisms [72]. 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 [73].

In the study by Rozich and Colvin [74] it was found that the presence of glucose attenuated the rate of phenol removal by phenol consuming cells. 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. Loh and Wang [19] reported that glucose supports growth and the addition of this conventional carbon source substantially increases cell density. Similarly, Topp *et al.* [75] showed that the addition of non-toxic compounds may stimulate the viability of cells and enhance degradation. 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.

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 cosubstrate for the microbial degradation of wide variety of organic chemicals; including hydrocarbons and aromatic ring compounds [76]. 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 [77] and respiration rate [78]. 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 [79]. Longmuir [78] reported critical dissolved oxygen concentrations in the range of 0.01 to 0.038 mg/L for a variety of yeast and bacterial cultures. For flocculant microbial cultures, the critical dissolved oxygen concentration is

generally higher and is typically in the range of 0.5 mg/L [79]. Above these critical oxygen levels, increases in oxygen concentration have little effect on microbial respiration rates.

IMMOBILIZED CELLS FOR PHENOL DEGRADATION

Phenol biodegradation has been studied in detail using pure and mixed cultures of suspended bacteria. However, at high concentrations of phenol inhibits microbial growth [57, 68]. Several strategies have been proposed to overcome substrate inhibition. These include cell acclimation to higher concentrations of phenol [70], the use of genetically engineered microorganisms [72] and cell immobilization [71].

The entrapment of biological agents in a gel matrix [80, 81] is quite effective, but several factors affect the specific activity of the immobilized biocatalyst when compared with free cells in suspension. Cell immobilization 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. 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 [82].

The degradation of aromatic compounds by immobilized cells has been reported and much work has been carried out on immobilized cell reactors using phenol as the model toxic compound [83, 84]. 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 [80]. The advantages of immobilized cells in comparison with suspended ones include the retention in the reactor of higher concentrations of microorganism, protection of cells against toxic substances and prevention of suspended bacterial biomass in the effluent [85]. In addition to this, immobilization of microbial cells provides high degradation efficiency and good operational stability [86] and immobilization appeared too as a promising procedure in overcoming substrate inhibition of phenol concentrations greater than 1000 mg/L [71].

Immobilized cells of *Pseudomonas putida* have been used successfully to degrade phenol at concentrations ranging from 100 to 1200 ppm in membrane and airlift bioreactors operated in batch and continuous mode [87, 88]. Also cells of *Pseudomonas aeruginosa* adsorbed on diatomaceous earth pellets (celite R-635) and packed in column bioreactors were used to degrade phenol up to 1200 ppm in inorganic defined medium [89]. Phenol biodegradation by suspended cells of *Rhodococcus* species P1 in continuous culture systems [90], *Rhodococcus* species immobilized in calcium alginate beads or on granular activated carbon [91] and immobilized *Rhodococcus erythropolis* UPV-1 in air stirred reactor [92] have been reported.

The concentration of sodium alginate commonly used in the preparation of immobilized cells affects the properties of the gel. However, little research has been performed on the effect of sodium alginate concentration on the degradation of phenol. In a free cell system, cell density will be relatively homogeneous in the medium and cell number in that homogeneity will be determined by substrate concentration. In immobilized cell systems, cell number will also be determined by substrate concentration but the distribution of cells is determined by the number of immobilized aggregations such as beads.

HEALTH EFFECTS

As the phenol present in the industrial effluents are toxic in nature and causes various health hazards (Table 4). Exposure to phenol by any route can produce systemic poisoning. Phenol is corrosive and causes chemical burns at the contact site. When exposed to skin it causes necrosis, symptoms of systemic poisoning often involve an initial, transient CNS stimulation, followed rapidly by CNS depression. Coma and seizures can occur within minutes or may be delayed up to 18 hours after exposure. Other symptoms include nausea, vomiting, diarrhea, methemoglobinemia, hemolytic anemia, profuse sweating, hypotension, arrhythmia, pulmonary edema, and tachycardia. In cardiovascular system it causes cardiac arrhythmias. Phenol exposure causes initial blood pressure elevation, then progressively severe low blood pressure and shock. Cardiac arrhythmia and bradycardia have also been reported following dermal exposure to phenol. Children's are more vulnerable to toxicants absorbed through the skin.

Route of exposure:

Inhalation

Phenol is absorbed rapidly from the lungs. However, because of its low volatility, inhalation hazard is limited. Phenol vapor is heavier than air. Children exposed to the same levels of phenol vapor as adults may receive larger doses because they have greater lung surface area: bodyweight ratios and increased minute volumes: weight ratios. Children may be more vulnerable to corrosive agents than adults because of the relatively smaller diameter of their airways. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of phenol vapor found nearer to the ground.

Table 4. Effect of phenol on various metabolic processes

Central nervous System	Initial signs and symptoms may include nausea, excessive sweating, headache, dizziness, and ringing in the ears. Seizures, loss of consciousness, coma, respiratory depression, and death may ensue. Coma and seizures usually occur within minutes to a few hours after exposure but may be delayed up to 18 hours.
Cardiovascular	Phenol exposure causes initial blood pressure elevation, then progressively severe low blood pressure and shock. Cardiac arrhythmia and bradycardia have also been reported following dermal exposure to phenol.
Respiratory	Mild exposure may cause upper respiratory tract irritation. With more serious exposure, swelling of the throat, inflammation of the trachea, tracheal ulceration, and an accumulation of fluid in the lungs can occur. Ingestion may lead to death from respiratory failure.
Gastrointestinal	Nausea, vomiting, abdominal pain, and diarrhea are common symptoms after exposure to phenol by any route. Ingestion of phenol can also cause severe corrosive injury to the mouth, throat, esophagus, and stomach, with bleeding, perforation, scarring, and orstricture formation as potential sequelae.
Renal	Renal failure has been reported in acute poisoning. Urinalysis may reveal the presence of protein (i.e., albuminuria), casts, and a green to-brown discoloration of the urine.
Hematologic	Components of the blood and blood-forming organs can be damaged by phenol. Most hematological changes (e.g., hemolytic, methemoglobinemia, bone marrow suppression, and anemia) can be detected by blood tests or simply by the color or appearance of the blood. Methemoglobinemia is a concern in infants up to 1 year old. Children may be more vulnerable to loss of effectiveness of Hemoglobin because of their relative anemia compared to adults.
Ocular	Contact with concentrated phenol solutions can cause severe eye damage including clouding of the eye surface, inflammation of the eye, and eyelid burns.
Dermal	When phenol is applied directly to the skin, a white covering of precipitated protein forms. This soon turns red and eventually sloughs, leaving the surface stained slightly brown. If phenol is left on the skin, it will penetrate rapidly and lead to cell death and gangrene. If more than 60 square inches of skin are affected, there is risk of imminent death. Phenol appears to have local anesthetic properties and can cause extensive damage before pain is felt.
Potential Sequelae	Chronic nerve damage has been reported due to acute exposure. Chemical burns may result in chronic skin and eye effects. Phenol ingestion may lead to narrowing of the esophagus, and cardiac and renal damage.
Carcinogenicity	Phenol has not been classified for carcinogenic effects. Phenol is, however, a known promoter of tumors.
Reproductive and developmental effects	No studies were located concerning the developmental or reproductive effects of phenol in humans. Animal studies have reported reduced fetal body weights, growth retardation, and abnormal development in the offspring of animals exposed to phenol by the oral route. Decreased maternal weight gain and increased maternal mortality were also observed.

Skin/Eye contact

All forms of phenol cause irritation and acute toxic effects of phenol most often occur by skin contact. Even dilute solutions (1% to 2%) may cause severe burns if contact is prolonged. Phenol vapor and liquid penetrate the skin with absorption efficiency approximately equal to the absorption efficiency by inhalation.

Metabolism

After oral uptake of phenol, there is a large first-pass metabolism. It is unclear whether phenol also undergoes first pass pulmonary metabolism and there have been conflicting results. The liver, lungs and the gastrointestinal mucosa are the most important sites of phenol metabolism. Conjugation with glucuronic acid to phenyl glucuronide and sulphation to phenyl sulphate, have been shown to be the

major metabolic pathways in several species. A shift from sulphation to glucuronidation was observed in rats after increasing the phenol doses, which is thought to be due to a saturation of the overall sulphation process, by the limited availability of 3-phosphoadenosine-5-phosphosulfate. The formation of sulphate and glucuronic metabolites occurs in the hepatocytes, and then transported to the bile or back into the blood. *In vitro* studies have shown the formation of the reactive metabolites 4, 4'-biphenol and diphenoquinone by neutrophils and activated leukocytes. Both *in vivo* and *in vitro* tests have shown covalent binding of phenol to tissue and plasma proteins and some phenol metabolites also bind to proteins.

Elimination and excretion

Urinary (renal) excretion is the major route of phenol elimination in animals and humans. The rate of excretion varies with different species, dose and route of administration. Three men after an oral administration of 0.01 mg/kg phenol excreted 90% of the dose in the urine within 24 hours, mainly as phenyl sulfate and phenyl glucuronide. Urinary excretion of humans exposed to phenol vapour via inhalation or skin, occurred with an excretion rate constant 0.2/hour. On oxidation to quinones the metabolites may tint the urine green. The half life is estimated to be between 1 and 4.5 hours with 52% eliminated unchanged in the urine. The natural presence of phenols in food and drug metabolites makes biological monitoring impossible. A minor part is eliminated in expired air and faeces.

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. Practical application of microorganisms for the degradation of phenol is 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.

REFERENCES

- [1] Hopkins, G.D. & McCarty, P.L. (1995). Field evolution of *In situ* aerobic co-metabolism of trichloroethylene and three dichlorotethylene isomers using phenol and toluene as the primary substrates. *Environ. Sci. Technol.*, 29:1628-1637.
- [2] Gabriele Pinto., Antonino Pollio., Lucio Previtiera. & Fabio Temussi. (2002). Biodegradation of phenol by Microalgae. *Biotechnol. Lett.*, 24:2047-2051.
- [3] Lika, K. & Papadakis, I.A. (2009). Modeling the biodegradation of phenolic compounds by microalgae. *J. Sea Res.*, 62(2-3):135-146.
- [4] Lee M.D., Thomas, J.M., Borden, R.C., Bedient, P.B., Ward, C.H. & Wilson, J.T. (1988). Bioremediation of aquifers contaminated with organic compounds. *CRC Crit. Rev. Env. Cont.*, 18:29-89.
- [5] Harwood, C.S. & Parales, R.E. (1996). The β -Ketoacid Pathway and the Biology of Self-Identity. *Ann. Rev. Microbiol.*, 50:553-590.
- [6] Stanier, R.Y. & Ornston, L.N. (1973). The β -Ketoacid Pathway. *Adv. Microbiol. Physiol.* 9:89-151.
- [7] Evans, W.C. (1947). Oxidation of phenol and benzoic acid by some soil bacteria. *J. Biol. Chem.*, 41:373-382.
- [8] Kilby, B.A. (1948). The Bacterial oxidation of Phenol to β -Ketoacid. *Proc. Biochem. Soc. Biochem. J.*, 43, V-Vi.
- [9] Williams, R.J. & Evans, W.C. (1975). The metabolism of Benzoate by *Moraxella* sp. Through Anaerobic Nitrate Respiration. *Biochem. J.*, 148:1-10.
- [10] Berfield, L.D. & Randall, CW. (1980). Biological process Design for waste water treatment. Prentice Hall, New Jersey.
- [11] Vladimir Bales. & Monika Antosova. (1999). Mathematical and experimental modeling of phenol degradation in air-lift bioreactor. *Environ. Engg and Policy.*, 1:209-216.
- [12] Davis, J.S. & Westlake, D.W.S. (1978). Crude oil utilization by fungi. *Can. J. Microbiol.*, 25:146-156.
- [13] Martin Hofrichter., Thomas Gunther. & Wolfgang Fritsche. (1992). Metabolism of phenol, chloro- and nitrophenols by the *Penicillium* strain Bi 7/2 isolated from a contaminated soil. *Biodegradation.*, 3:415-421
- [14] Anselmo, A.M. & Novais, J.M. (1984). Isolation and selection of phenol degrading microorganisms from an industrial effluent. *Biotechnol. Lett.*, 9:601-606.

- [15] Valli, K. & Gold, M.H. (1991). Degradation of 2, 4-dichlorophenol by the lignin-degrading fungus *Phanerochaete chrysosporium*. *J. Bacteriol.*, 173:345-352.
- [16] Kennes, C. & Lema, J.M. (1994). Simultaneous biodegradation of p-cresol and phenol by the basidiomycete *Phanerochaete chrysosporium*. *J. Ind. Microbiol.*, 13:311-314.
- [17] Yong Lu., Lianne Yan., Ying Wang., Shenfan Zhou., Jiajun Fu. & Jianfa Zhang. (2009). Biodegradation of phenolic compounds from coking waste water by immobilized white rot fungus *Phanerochaete chrysosporium*. *J. Hazard. Mater.*, 165(1-3):1091-1097.
- [18] Farrell, A. & Quilty, B. (1999). Degradation of Monochlorophenols by a mixed microbial community via a meta-cleavage pathway. *Biodegradation.*, 10:352-362.
- [19] Loh, K.C. & Wang, S.J. (1998). Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation.*, 8:329-338.
- [20] Yan Jiang., Jianping Wen., Jing Bai., Xiaoqiang Jia. & Zongding Hu. (2007). Biodegradation of phenol at high initial concentration by *Alcaligenes faecalis*. *J. Hazard. Mater.*, 147:672-676.
- [21] Sung Ho Yeom., Seung Ho kim., Young Je Yoo. & In Sang Yoo. (1997). Microbial adaptation in the degradation of phenol by *Alcaligenes xylosoxidans* Y234. *Korean J. Chem. Eng.*, 14:37-40.
- [22] Kar, S., Swaminathan, T. & Baradarajan, A. (1997). Biodegradation of phenol and cresol isomer mixtures by arthrobacter. *World J. Microbiol. Biotechnol.*, 13:659-663.
- [23] Chandrakant Karigar., Aravind Mahesh., Manjunath Nagenahalli. & Dae Jin Yun. (2005). Phenol degradation by Immobilized cells of *Arthrobacter citreus*. *Biodegradation.*, 17:47-55.
- [24] Maria Unell., Karolina Nordin., Cecilia Jernberg., John Stenstrom. & Janet, K Jansson. (2007). Degradation of mixtures of phenolic compounds by *Arthrobacter chlorophenolicus* A6. *Biodegradation.*, 19:495-505.
- [25] Gurusamy Annadurai., Lai Yi Ling. & Jiunn-Fwu Lee. (2008). Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. *J. Hazard. Mater.*, 151:171-178.
- [26] Bandyopadhyay, K., Das, D. & Maiti, B.R. (1998). Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. *Bioprocess Eng.*, 18:373-377.
- [27] Folsom, B.R., Chapman, P.J. & Pritchard, P.H. (1990). Phenol and Trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. *Appl. Environ. Microbiol.*, 56(5):1279-1285.
- [28] Gurusamy Annadurai., Lai Yi Ling. & Jiunn-Fwulee. (2007). Biodegradation of phenol by *Pseudomonas pictorum* on immobilized with chitin. *African J. Biotechnol.*, 6(3):296-303.
- [29] Kotresha, D. & Vidyasagar, G.M. (2008). Isolation and characterization of phenol-degrading *Pseudomonas aeruginosa* MTCC 4996. *World J. Microbiol. Biotechnol.*, 24:541-547.
- [30] Ahmed, A.M. (1995). Phenol degradation by *Pseudomonas aeruginosa*. *J. Environ. Sci. Health.*, 30:99-103.
- [31] Song, H., Liu, Y., Xu, W., Zeng, G., Aibibu, N., Xu, L. & Chen, B. (2009). Simultaneous Cr (VI) reduction and phenol degradation in pure cultures of *Pseudomonas aeruginosa* CC7CCAB91095. *Bioresour. Technol.*, 100(21):5079-5084
- [32] Wurster, M., Mundt, S., Hammer, E., Schauer, F. & Lindequist, U. (2003). Extracellular degradation of phenol by the *Cyanobacterium synechococcus* PCC 7002. *J. Appl. Phycol.*, 15:171-176.
- [33] Tallur, P.N., Megadi, V.B. & Nin ekar, H.Z. (2008). Biodegradation of p-cresol by Immobilized cells of *Bacillus* sp. Strain PHN1. *Biodegradation.*, 20:79-83.
- [34] Arutchelvan, V., Kanakasabai, V., Elangovan, R., Nagarajan, S. & Muralikrishnan, V. (2006). Kinetics of high strength phenol degradation using *Bacillus brevis*. *J. Hazard. Mater.*, 129:216-222.
- [35] Sunil S Adav., Ming-Yuan Chen., Duu-Jong Lee. & Nan-Qi Ren. (2007). Degradation of phenol by Aerobic Granules and Isolated Yeast *Candida tropicalis*. *Biotechnol. Bioeng.*, 96:844-852.
- [36] Yan Jiang., Jianping Wen., Li Lan. & Zongding Hu. (2007). Biodegradation of phenol and 4-chlorophenol by the yeast *Candida tropicalis*. *Biodegradation.*, 18:719-729.
- [37] Varma, R.J. & Gaikwad, B.G. (2009). Biodegradation and phenol tolerance by recycled cells of *Candida tropicalis* NCIM 3556. *Int. Biodeterior. Biodegrad.*, 63(4):539-542
- [38] Rita J Varma. & Bhaskar G Gaikwad. (2008). Rapid and high biodegradation of phenols catalyzed by *Candida tropicalis* NCIM 3556 cells. *Enzyme Microb. Technol.*, 43(6):431-435
- [39] Weijian Cai., Jiwu Li. & Zhen Zhang. (2007). The characteristics and mechanisms of phenol biodegradation by *Fusarium* sp. *J. Hazard. Mater.*, 148:38-42.
- [40] Vera L Santos., Nadia, M., Heilbuth, Danubia, T., Braga, Andrea, S., Monteiro, Valter, R. & Linardi, Dr. (2002). Phenol degradation by a *Graphium* sp. FIB4 isolated from industrial effluents. *J. Basic Microbiol.*, 43:238-248.
- [41] Krik, T., Semplea. & Ronald B Caina. (1997). Degradation of phenol and its methylated homologues by *Ochromonas danica*. *FEMS Microbiol. Lett.*, 152:133-139.
- [42] Stoilova, I., Krastanov, A., Yanakieva, I., Kratchanova, M. & Yemendjiev, H. (2007). Biodegradation of mixed phenolic compounds by *Aspergillus awamori* NRRI 3112. *Int. Biodeterior. Biodegrad.*, 60:342-365.
- [43] Ahmadi, M., Vahabzadeh, F., Bonakdarpour, B., Mehranian M. & Mofarrah, E. (2005). Phenolic removal in live oil mill waste water using loofah-immobilized *Phanerochaete chrysosporium*. *World J. Microbiol. Biotechnol.*, 22:119-127.
- [44] Begona Prieto, M., Aurelio Hidalgo., Juan L serra. & Maria J Llama. (2002). Degradation of phenol by *Rhodococcus erythropolis* UPV-1 immobilized on Biolite in a packed-bed reactor. *J. Biotechnol.*, 97:1-11.
- [45] Krallish Irina., Gonta Svetlana., Savenkova, Ludmila., Bergauer Phillip. & Margesin Rosa. (2006). Phenol degradation by immobilized cold-adapted yeast strains of *Cryptococcus terreus* and *Rhodotorula creatinivora*. *Extremophiles.*, 10:441-449.

- [46] Bielefeldt, A.R. & Cort, T. (2005). Dual substrate biodegradation of a Non-ionic surfactant and Pentachlorophenol by *Sphingomonas chlorophenolica* RA2. *Biotechnol. Bioeng.*, 89(6):680-689.
- [47] Vera L santose., Nadia M Heilbuth., Valter, R. & Linardi Dr. (2001). Degradation of phenol by *Trichosporon* sp. LE3 cells immobilized in alginate. *J. Basic Microbiol.*, 41:171-178.
- [48] Aleksieva, Z., Ivanova, D., Godjevargova, T. & Atanasov, B. (2002). Degradation of some phenol derivatives by *Trichosporon cutaneum* R57. *Process Biochem.*, 37:1215-1219.
- [49] Holladay, D.W., Chilcote, D.D., Hancher, C.W. & Scott, C.D. (1978). Biodegradation of phenolic waste liquors in Stirred Tank, Packed-Bed and Fluidized-Bed Bioreactors. *J. Water Poll. Control Federation.*, 50:2573-2578.
- [50] Sung Ho Yeom., Seung Ho Kim., Young Je Yoo. & In Sang Yoo. (1997). Microbial adaptation in the degradation of phenol by *Alcaligenes xylosoxidans* Y234. *Korean J. Chem. Eng.*, 14:37-40.
- [51] Yi Li. & Kai-Chee Loh. (2007). Continuous Phenol Biodegradation at High Concentrations in an Immobilized-Cell Hollow Fiber Membrane Bioreactor. *J. Appl. Polym. Sci.*, 105:1732-1739.
- [52] Sameer H Israni., Shrikant S Koli., Ashwin W Patwardhan., Melo, J.S. & Dsouza, S.F. (2002). Phenol degradation in rotating biological contactors. *J. Chem. Technol. Biotechnol.*, 77:1050-1057.
- [53] Hossein Nikakhtari. & Gordon A Hill. (2006). Continuous bioremediation of phenol-polluted air in an external loop airlift bioreactor with a packed bed. *J. Chem. Technol. Biotechnol.*, 81:1029-1038.
- [54] Vidya Shetty, K., Ramanjaneyulu, R. & Srinikethan, G. (2007). Biological phenol removal using immobilized cells in a pulsed plate bioreactor: Effect of dilution rate and influent phenol concentration. *J. Hazard. Mater.*, 149:452-459.
- [55] Hughes, S.M. & Cooper, D.G. (1996). Biodegradation of phenol using the Self Cycling Fermentation (SCF) Process. *Biotechnol. Bioeng.*, 51:112-119.
- [56] Chao Wang. & Yi Li. (2007). Incorporation of granular activated carbon in an immobilized membrane bioreactor for the biodegradation of phenol by *Pseudomonas putida*. *Biotechnol. Lett.*, 29:1353-1356.
- [57] Hill, G.A. & Robinson, C.W. (1975). Substrate inhibition kinetics: phenol degradation by *Pseudomonas putida*. *Biotechnol. Bioeng.*, 17:1599-1615.
- [58] Kim, N.W. & Armstrong, M.E. (1981). A comprehensive study on the biological treatabilities of phenol and methanol II. The effects of temperature, pH, salinity and nutrients. *Water Res.*, 15:1233-1247.
- [59] Robertson, B.K. & Alexander, M. (1992). Influence of calcium iron and pH on phosphate availability for microbial mineralization of organic chemicals. *Appl. Environ. Microbiol.*, 58:38-41.
- [60] Chitra. (1995). Studies on biodegradation of phenolic compounds by *Pseudomonas pictorum*. PhD thesis CLRI. University of Madras, Chennai-25.
- [61] Annadurai, G., Mathalai Balan, S. & Murugesan, T. (1999). Box-Behnken design in the development of optimized complex medium for phenol degradation using *Pseudomonas putida* (NICM 2174). *Bio process Eng.*, 21:415-421.
- [62] Annadurai, G., Rajesh Babu, S., Mahesh, K.P.O. & Murugesan, T. (2000). Adsorption and biodegradation of phenol by chitosan-immobilized *Pseudomonas putida* (NICM 2174). *Bio process Eng.*, 22:493-501.
- [63] Pakula, A., Bieszkiewicz, E., Boszczyk Maleszak, H. & Mycielski, R. (1999). Biodegradation of phenol by bacterial strains from petroleum refining wastewater purification plant. *Acta Microbiol Pol.*, 48:373-380.
- [64] Annadurai, G., Juang, R.S. & Lee, D.J. (2002). Microbiological degradation of phenol using mixed liquors of *Pseudomonas putida* and activated sludge. *Waste Manage.*, 22:703-710.
- [65] Paraskevi, N.P. & Euripides, G.S. (2005). Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil *Pseudomonad*. *Biodegradation.*, 16:403-413.
- [66] Ratkowsky, D.A., Olley, J., Mc Meekin, T.A. & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.*, 149:1-5.
- [67] Allsop, P.J., Chisti, Y., Moo-Young. & Sullivan, G.R. (1993). Dynamics of phenol degradation by *Pseudomonas putida*. *Biotechnol. Bioeng.*, 41:572-580.
- [68] Wang, S.J. & Loh, K.C. (1999). Modeling the role of metabolic intermediates in kinetics of phenol biodegradation. *Enzyme Microb. Technol.*, 25:177-184.
- [69] Sala-Trepat, J.M., Murray, K., & Williams, P.A. (1972). The metabolic divergence in the meta cleavage of catechols by *Pseudomonas putida* NCIB 10015. *Eur. J. Biochem.*, 28:347-356.
- [70] Carme Masque., Maite Nolla. & Albert Bordons. (1987). Selection and adaptation of a phenol degrading strain of *Pseudomonas*. *Biotechnol. Lett.*, 9:655-660.
- [71] Loh, K.C., Chung, T.S. & Ang, Y.W.F. (2000). Immobilized cell membrane bioreactor for high strength phenol wastewater. *J. Environ. Eng.*, 126:75-79.
- [72] Soda, S., Ike, M. & Fujita, M. (1998). Effects of inoculation of genetically engineered bacterium on performance and indigenous bacteria of sequencing batch activated sludge process treating phenol. *J. Ferment. Bioeng.*, 86:90-96.
- [73] Armenante, P.M., Fabio Fava. & David Kafkewitz. (1995). Effect of Yeast extract on growth kinetics during aerobic biodegradation of chlorobenzoic acids. *Biotechnol. Bioeng.*, 47: 227-233.
- [74] Rozich, A.F. & Colvin, R.J. (1985). Effects of glucose on phenol biodegradation by heterogeneous populations. *Biotechnol. Bioeng.*, 28:965-971.
- [75] Topp, E. & Hanson, R.S. (1988). Degradation of pentachlorophenol by a *Flavobacterium* species grown in continuous culture under various nutrients limitations. *Appl. Environ. Microbiol.*, 54:2452-2459.
- [76] Dagley, S. (1972). Microbial degradation of stable chemical structures: general features of metabolic pathways. In: Degradation of synthetic organic molecules in the biosphere. National Academy of Sciences, Washington, DC, p.1-16.
- [77] Button, D.K. & Garver, J.C. (1966). Continuous culture of *Torulopsis utilis*: a kinetic study of oxygen limited growth. *J. Gen. Microbiol.*, 45:195-204.

- [78] Longmuir, I.S. (1954). Respiration rate as a function of oxygen concentration. *Biochem. J.*, 57:81-87.
- [79] Gaudy, A.F., Jr. & Gaudy, E.T. (1980). Microbiology for environmental scientists and engineers. McGraw-Hill Book Co., New York.
- [80] Bettman, H. & Rehm, H.J. (1984). Degradation of phenol by polymer entrapped microorganisms. *Appl. Environ. Microbiol.*, 20:285-290.
- [81] Scott, C.D. (1987). Immobilized cells, a review of recent literature. *Enzyme Microb. Technol.*, 9:66-73.
- [82] Alexieva, Z., Gerginova, M., Zlateva, P. & Pnenva, N. (2004). Comparison of growth kinetics and phenol metabolizing enzymes of *Trichosporon cutaneum* R57 and mutants with modified degradation abilities. *Enzyme Microb. Technol.*, 34:242-247.
- [83] Shin-Fan, L., Fujie, K., Long, T. & Tan, W.T. (1987). Characteristics of draft tube gas liquid-solid fluidized bed bioreactor with immobilized living cells for phenol degradation. *Biotechnol. Bioeng.*, 30:498-504.
- [84] Worden, R.M. & Donaldson, T.L. (1987). Dynamics of a biological fixed film for phenol degradation in a fluidized bed reactor. *Biotechnol. Bioeng.*, 30(3):398-412.
- [85] Ozaki, H., Liu, Z. & Terashima, Y. (1991). Utilization of microorganisms immobilized with magnetic particles for sewage and wastewater treatment. *Water Sci. Technol.*, 23:1125-1136.
- [86] Koch, B., Ostermann, M., Hoke, H. & Hempel, D.C. (1991). Sand and activated carbon as biofilm carriers for microbial degradation of phenols and nitrogen-containing aromatic compounds. *Water Res.*, 25:1-8.
- [87] Mordocco, A., Kuek, C. & Jenkins, R. (1999). Continuous degradation of phenol at low concentration using immobilized *Pseudomonas putida*. *Enzyme Microb. Technol.*, 25:530-536.
- [88] Muftah, H., El-Naas., Shaheen, A., Al-Muhtaseb. & Souzan Makhoulouf. (2009). Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *J. Hazard. Mater.*, 164(2-3):720-725.
- [89] Durham, D.R., Marshall, L.C., Miller, J.G. & Chmurny, A.B. (1994). Characterization of inorganic bio carriers that moderate system upsets during fixed-film biotreatment processes. *Appl. Environ. Microbiol.*, 60:3329-3335.
- [90] Hensel, J. & Straube, G. (1990). Kinetic studies of phenol degradation by *Rhodococcus* Sp. P1.II. Continuous cultivation. *Antonievan Leeuwenhoek.*, 57:33-36.
- [91] Pai, S.L., Hsu, Y.L., Chong, N.M., Sheu, C.S. & Chen, C.H. (1995). Continuous degradation of phenol by *Rhodococcus* sp. immobilized on granular activated carbon and in calcium alginate. *Bioresour. Technol.*, 51:37-42.
- [92] Prieto, M.B., Hidalgo, A., Rodriguez, C., Fernandez., Serra, J.L., & Llama, M.J. (2002). Biodegradation of phenol in synthetic and industrial waste water by *Rhodococcus erythropolis* UPV-1 immobilized in an air stirred reactor with clarifier. *Appl. Microbiol. Biotechnol.*, 58:853-859.

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