CHAPTER 2

LITERATURE REVIEW

2.1 Phenol

Petroleum hydrocarbons can be divided into four classes namely saturates, aromatics, asphaltenes (phenol, fatty acids, ketones, esters and porphyrines), and resins (pyridines, quinolines, carbazoles, sulfoxides and amides) (Colwell and Walker, 1977). Petroleum products have vast uses in this modern society. Phenol is an important industrial chemical of environmental concern widely used in many industries such as coke, refineries, manufacturers of resin, pharmaceuticals, pesticides, dyes, plastics, explosives and herbicides, and can also occur in their wastewaters (Yang *et al*., 1998; Bandhyopadhyay *et al*., 2001; Kumar *et al*., 2004). Phenols are produced in very large quantities for use as solvents, and starting materials for chemical synthesis (Budavari, 1996).

Phenols and its derivatives are some of the major hazardous compounds in industrial wastewater (Peters *et al*., 1997). For instance, phenol is released into water from industrial effluent discharges such as petroleum refinery wastewater, petrochemical plants, coking plants, olive mill wastewater and phenol resin industry

plants (Aksu, 2005; Yan *et al*., 2006; Bai *et al*., 2007). Phenol has been also detected in rivers, industrial effluents, and landfill runoff waters (Lee *et al*., 2006).

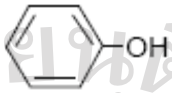
Phenols have relatively high water solubility and widely known to be acutely toxic to a range of organisms. It produces undesirable taste, odour, colour to water and is considered toxic (Klibanov, 1982). Therefore, this compound needs to be disposed off in a safe and environmentally acceptable way.

2.2 Phenol chemical and physical properties

Some chemical and physical properties of phenol (also called phenic acid or carbolic acid) are reported in Table 2.1. Phenol was first isolated from coal tar in 1834 by the German chemist Runge. It is an aromatic compound. At ambient temperature and pressure it is a hygroscopic crystalline solid. When pure, solid phenol is white but is mostly colored due to the presence of impurities. Phenol is very soluble in ethyl alcohol, in ether and in several polar solvents, as well as in hydrocarbons such as benzene. In water it has a limited solubility and behaves as a weak acid. As a liquid phenol attacks rubber, coatings, and some forms of plastic (Jordan *et al*., 2002; Nair *et al*., 2008). Hot liquid phenol attacks aluminum, magnesium, lead, and zinc metals. It is characterized by a typical pungent sweet, medicinal, or tar-like odour (Amore and Hautala, 1983). It is a combustible compound.

Table 2.1 Physical properties and chemical identity of phenol.

CAS Registry Number	108-95-2	Lide, 1993
Synonym(s)	Benzenol, hydroxybenzene, monophenol, oxybenzene, phenyl alcohol, phenyl hydrate, phenyl hydroxide	ATSDR, 1998
Registered trade name(s)	Carbolic acid, phenic acid, phenic alcohol	ATSDR, 1998
Melting point, ºC	43	Lide, 1993
Boiling point, ºC	181.8	Lide, 1993
Vapor pressure, at 25 ºC	0.3513	HSDB, 1998
Density, at 20 ºC relative to the density of H_2O at 4 ºC	1.0576	Lide, 1993
Flashpoint (open cup)	85 ºC	ATSDR, 1998
Water solubility, g/L at 25 ºC	87	Lide, 1993
Log KOW	1.46	HSDB, 1998
Odor threshold	0.047 ppm (0.18 mg/m^3) - 100% response 0.006 ppm (0.02 mg/m^3) - sensitive	U.S. EPA, 1986
Molecular weight	94.12	Calculated
Conversion factors	1 ppm (v/v) = mg/m3 x 0.260	Calculated

	1 mg/m3 = ppm (v/v) x 3.85	
Empirical formula	C_6H_6O	Lide, 1993
Chemical structure		Not applicable

Source : U.S. EPA, 2000

2.3 Sources of phenol

The origin of phenol in the environment is from natural, man-made and endogenous sources. Phenol is released primarily to the air and water as a result of its manufacture and use, wood burning and auto exhaust. Phenol mainly enters waters from industrial effluent discharges.

2.3.1 Natural sources

Phenol is a constituent of coal tar, and is formed during decomposition of organic materials. Increased environmental levels may result from forest fires (Hubble *et al*., 1981). It has been detected among the volatile components from liquid manure at concentrations of 7-55 µg/kg dry weight and has an average concentration in manure of 30 µg/kg dry weight (Spoelstra,1978).

2.3.2 Man-made sources

Man-made sources are from industrial wastes from fossil fuel extraction, chemical manufacturing processes such as phenol manufacturing plants, pharmaceutical industry, wood processing industry and pesticide manufacturing plants (Kumaran and Parachuri, 1997). Industrial sources of phenols and other related aromatics are from petroleum refinery, petrochemicals, basic organic chemical manufacture, coal refining, pharmaceuticals, tannery and pulp and paper mills (Table 2.2) (Kumaran and Paruchuri, 1997).

2.3.3 Endogenous sources

An important additional source of phenol may be the formation from various xenobiotics such as benzene (Pekari *et al*., 1992) under the influence of light (Hoshino and Akimoto, 1978).

Table 2.2 Sources of phenols and other related aromatic compounds in wastewater.

Sources	Significant phenolic compounds
Petroleum refining	Hydrocarbons (alkanes, cycloalkanes, polyaromatic hydrocarbons), benzenes, substituted benzenes, toluenes, *n*-octanes, *n*-decanes, naphthalenes, biphenyles**, phenol**, cyanide, sulphide and ammonia.
Petrochemicals	Naphthalene, hepatanes, benzenes, butadiene, C-4 alcohols, **phenol** and resorcinol.

Table 2.2 Sources of phenols and other related aromatic compounds in wastewater.

(continued)

Sources	**Significant phenolic compounds**
Basic organic chemical Manufacturing	*m*-amino **phenol**, resorcinol, dinitrophenol, p-nitrophenol, trinitrophenol, benzene sulphonic acids, aniline, chlorobenzenes, toluene and resorcinol.
Coal refining	**Phenol,** catechol, *o*-, *m*-, *p*-cresols, resorcinol, hydroquinone, pyrogallol, polyaromatic hydrocarbons, pyridine, pycolines, lutidines, xylenes, toluenes, benzoic acid.
Pharmaceuticals	Toluenes, benzyl alcohols, phenyl acetic acid, chlorinated products of benzene, chloroform, ether, ethyl alcohol.
Tannery	Tannin, catechin, **phenol**, chlorophenol, nitrophenols.
Pulp and paper mills	Lignin, vanillin, vanillic acid, dehydrodivanillin, ferulic acid, cinnamic acid, synringic acid, vieratric acid, protocatechuic acid, gentisic acid, benzoic acid, guadiachols, catechol, coniferyl alcohol, dehydrodihydroconiferyl alcohol, phenyl propionic acid, **phenols** and chlorophenols.

Source : Kumaran and Paruchuri, 1997

The annual production of phenols are estimated around 1.25 x 109 kg (Bechard *et al*., 1990). In 1995, the total annual capacity of phenol production approached 4.5 billion pounds. The most commonly used production method for phenol is from cumene (isopropylbenzene) (IARC, 1989). Phenol is also produced from chlorobenzene and toluene. It is the basic feedstock from which a number of

commercially important materials are made, including phenolic resins, bisphenol A (2,2-bis-1- hydroxyphenylpropane), capro-lactam, alkyl phenols, chlorophenols such as pentachlorophenol (IARC, 1989). Phenolic resins are used as a binding material in, insulation material, chipboard and triplex, paints and casting sand foundries. Phenols are environmental pollutants commonly present in the wastewaters from oil industry.

2.4 Release of phenol into the environment

Man-made phenolic compounds are found in the environment in abundance, due to agricultural and industrial activities. It has been reported that an estimated total of 23.5 million pounds (10.6 million kg) of phenol was released to the environment from 689 large processing facilities (TRI, 1998). Phenol has been found in surface water, ground water, soil and sediment (HazDat, 1998).

2.4.1 Air

The estimated releases of phenol of 9.5 million pounds (4.3 million kg) to air from 635 large processing facilities accounted for about 5% of environmental releases (TRI, 1998). During manufacturing, phenol is released to the atmosphere from storage tank vents during transport loading (Delaney and Hughes, 1979).

Other major sources of release to the atmosphere are from residential wood burning and automobile exhaust (Scow *et al*., 1981). Phenol has been detected in the exhaust gases of private cars at concentration of 0.3 ppm (approximately 1.2 mg/m3) to 1.4-2.0 ppm (5.4-7.7 mg/m3) (Kuwata *et al*., 1980; Verschueren, 1983).

Phenol has been detected from other sources such as emissions from waste incinerator plant at 0.36 ppb (Jay and Stieglitz, 1995), in cigarette smoke and plastics (Graedel, 1978). It has been identified in cigarette smoke, in quantities that are comparable to an average emission of 0.4 mg/cigarette (Groenen, 1978). Emission gases from all material incinerators and home fires, especially wood-burning, may contain substantial quantities of phenol (Den Boeft *et al.*, 1984).

Volatilization from environmental waters and soil has been shown to be a slow process and not expected to be a significant source of phenol in the atmosphere.

2.4.2 Water

It has been reported that an estimated of 72,550 pounds (32,650 kg) of phenol releases to water from 230 large processing facilities accounted for about 0.3% of total environmental releases (TRI, 1998).The most common anthropogenic sources of phenol in natural water include coal tar and waste water from manufacturing industries such as resins, plastics, fibers, adhesives, iron, steel, aluminum, leather, rubber, and influents from synthetic fuel manufacturing (Parkhurst *et al*., 1979).

Phenol is also released from paper pulp mills (Keith, 1976) and wood treatment facilities (Goerlitz *et al*., 1985). Phenol has been detected in the effluent discharges of a variety of industries. Levels of phenol concentration in wastewater from selected industries are shown in Table 2.3.

Table 2.3 Typical levels of phenol concentration in wastewater of some selected Industries.

Selected industry	**Phenol concentration (mg/l)**	**Reference**
Phenol production	3,000-4000	Godjevargova *et al*., 2003
Pulp and paper	33.1-40	Peralta-Zamora *et al*., 1998; Minussi *et al*., 1998
Textile	12.3	Kunz *et al*., 2001
Olive oil mill	3000-10,000	Knupp *et al*., 1996; Robards and Ryan, 1998
Coal conversion plant	4-4780	Parkhurst *et al*., 1979
Shale oil wastewater	4.5	Hawthorne and Sievers, 1984
Ash-heap water (oil shale)	500	Kahru *et al*., 1998
Phenolic resins production	1200->10,000	Patterson, 1985; Kavitha and Palanivelu, 2004
Methyl violet and cumene-Phenol production	310-660	Kanekar *et al*., 1999
Chemical specialities-Petroleum oil refinery	33.5	Pfeffer, 1979

Other release of phenol results from commercial use of phenol and phenol containing products, including slimicides, general disinfectants (Budavari *et al*., 1989), medicinal preparations such as ointments, ear and nose drops, cold sore

lotions, mouthwashes, gargles, toothache drops, analgesic rubs, throat lozenges (USEPA, 1980), and antiseptic lotions (Musto *et al*., 1977). It has been estimated that 3.8 kg/day of phenol release to seawater from municipal treatment facilities (Crawford *et al*., 1995). Animal and decomposition of organic wastes are the two natural sources of phenol in aquatic media.

2.4.3 Soil

In 1996, the estimated releases of 159,059 (71,577 kg) of phenol to soil from 102 large processing facilities accounted for about 0.7% of total environmental releases (TRI, 1998). Phenol are released to the soil during its manufacturing process, loading and transport when spills occur, and when it leaches from hazardous wastes sites and landfills (Xing *et al*., 1994). According to ASTDR, (1998) generally the data on concentrations of phenol found in soil at sites other than hazardous sites are lacking. This may be due to a rapid rate of biodegradation and leaching. Phenol can be expected to be found in soils that receive continuous or consistent releases from a point source. Phenol that leaches through soil to groundwater spends at least some time in that soil as it travels to the groundwater. Phenol has been found in groundwater, mainly at or near hazardous wastes sites.

2.5 Hazards of phenol

Aromatic hydrocarbons are not as readily biodegradable as the normal and branched alkanes, they are somewhat more easily degradable than the alicyclic

hydrocarbons (Leahy and Colwell, 1990). Many of these compounds are toxic and some are known or suspected carcinogens (Sheeja and Murugesan, 2002). The presence of phenol in drinking water and irrigation water represents a serious health hazards to humans, animals, plants and microorganisms (Jiang *et al*., 2002).

Phenol concentrations greater than 50 ppb are toxic to some form of aquatic life and ingestion of 1 g of phenol can be fatal in human beings (Seetharam and Saville, 2003). Continuous ingestion of phenol for a prolonged period of time causes mouth sore, diarrhea, excretion of dark urine and impaired vision at concentrations levels ranging between 10 and 240 mg/l (Barker *et al*., 1978). Lethal blood concentration for phenol is around 4.7 to 130 mg/100 ml. Phenol affects the nervous system and key organs, i.e. spleen, pancreas and kidneys (Manahan, 1994).

Phenol is lethal to fish even at relatively low levels, e.g. 5-25mg/L, depending on the temperature and state of maturity of rainbow trout (Brown *et al*., 1967). Phenolic compounds are also responsible for several biological effects, including antibiosis (Gonzalez *et al*., 1990), ovipositional deterrence (Girolami *et al*.,1981) and phytotoxicity (Capasso *et al*., 1992).

Phenol is classified as a priority pollutant owing to their high toxicity and wide spread environmental occurrence (USEPA, 2000). Various regulatory authorities have imposed strict limits to phenol concentration in industrial discharges. Phenol is released into the environment is regulated by many countries (Sa and Boaventura, 2001). For drinking waters, it has been prescribed a guideline concentration of 1 μg/l

(WHO, 1994). In Malaysia, the Environmental Protection Act, 1974 establish a phenol concentration of 0.001 mg/l for Standard A, 0.1 mg/l for standard B, and 5 mg/l other than standard A and B as the limit for wastewater discharges into inland waters. Therefore, the disposal of phenol has become a major global concern (Percival and Senior, 1998).

2.6 Process for treating phenol

The available techniques for treating phenol can be subdivided into two main groups, *i.e.* the destruction and recovery methods (Saxena and Jotshi, 1996). Among the destruction methods, the most commonly employed are biological treatments (Ryan *et al*., 2005; Bai *et al*., 2007), incineration, ozonation in the presence of UV radiation and oxidation with wet air (Saxena and Jotshi, 1996). The recovery methods include adsorption and electroadsorption onto charcoal (Jain *et al.*, 2002), ion exchange resins (Palepu *et al.*, 1995), membrane processes such as pervaporation (Pradhan *et al.*, 2002), extraction with membrane (Boam and Zhang, 2001), supported liquid membrane (Kujawski *et al.*, 2004) and liquid membrane in emulsion (Lin *et al.*, 1999).

2.6.1 Chemical methods

Chemical oxidation, using O_3, ClO_2, Cl_2, and H_2O_2 as oxidants, is a popular method (Yurii and Moshe, 1998). However, it is prohibitively expensive if large volumes are to be treated, particularly if the waste is predominantly organic.

In view of this, chemical treatment is usually used as a pretreatment step for removing color and some toxic compounds so that other treatment methods, such as biological treatment, can be used downstream (Yurii and Moshe, 1998). UV/O_3 oxidation allows the combination of photocatalysts and UV light to achieve higher reaction rates in aqueous streams, but the ozone and UV lighting requirements can be expensive as well as difficult to scale up to industrial sized reactors (Matthews, 1992).

2.6.2 Physical methods

As a physical treatment method, an adsorptive process over activated carbon (and some synthetic resins) is used frequently to treat wastewater (Efremenko and Sheintuch, 2006). High-molecular-weight organics (particularly those having lower solubility in water) are adsorbed preferentially on the carbon surface. Carbon adsorption treatment becomes attractive if the spent carbon can be regenerated by biotreatment or solvent extraction (Figueroa and Weber, 2005). Reverse osmosis is a membrane process used for desalting brackish water and removing dissolved solids from certain industrial wastewaters (Goncharuk *et al*., 2002). However, the technique is not used frequently primarily due to high membrane replacement costs (Matthews, 1992).

2.6.3 Biological methods

Biodegradation is versatile, inexpensive and can potentially turn a toxic material into harmless products. If properly designed and operated, biological

processes can realize total oxidation of organic matter so that there can be no sludge's that must be eradicated as a result of treatment.

Removal of phenol from wastewaters can be achieved through aerobic biodegradation in well-run activated sludge plants (Symons and McKinney, 1995). A *Pseudomonas* sp. strain capable of degrading pentachlorophenol was isolated from PCP-contaminated soils and identified as *Pseudomonas mendocina* NSYSU (Kao *et al*., 2005). *Cyanobacteria* are in more advantageous position than heterotrophic bacteria because of their trophic independence for nitrogen as well as carbon (Suseela *et al.*, 1991). A pure culture of *Pseudomonas putida* was grown in both batch and continuous cultures using phenol as the limiting substrate. Phenol was effectively removed and degraded by the marine *Cyanobacterium phormidium valderianum* (Kirchner *et al.*, 2003). Although bacteria are most likely to be responsible for aerobic breakdown of phenol in activated sludge, fungi including *Trichosporon cutaneum* and *Candida tropicalis* cells are also capable of utilizing phenol as the major carbon source (Komárková and Páca, 2000; Jia *et al*., 2006; Galındez-Mayer *et al*., 2008).

2.7 Microbial degradation

Microbial degradation of chemicals in the environment is a route for their removal. The microbial degradation of these compounds is a complex series of biochemical reactions and often different when different microorganisms are involved. The interdependence of biodegradation, biotransformation and biocatalysis

has been reviewed by Parales *et al*. (2002). Microbial degradation of pollutants is crucial in order to predict their longevity and long term effects and also important in the actual remediation process (Landis and Yu, 2003).

In aerobic respiration, oxygen acts as the electron acceptor. Molecular oxygen is a reactant for oxygenase enzymes and is incorporated into the final products. In anaerobicrespiration, different inorganic electron acceptors are possible such as NO_3, SO_4^{2-}, SO, CO_2 and Fe^{3+}. Most of the documentation on microbial degradation of organic pollutants in nature is focused on aerobic transformation. Many synthetic compounds accumulate in nature because the release rates exceed the rates of microbial and chemical degradation (Harms and Bosma, 1997). In addition, many microbial transporters and catabolic are regulated, *i.e.* they are only synthesizes in response to the presence of a certain concentration of their substrate (Spain and van Veld, 1983).

There are two major reasons for low degradation rates which have been identified. First, the biochemical potential to degrade certain compound is limited. This is more likely that less chemicals resemble natural compounds (Van deer Meer *et al*., 1992). Secondly, the pollutant or other substrates, *e.g.* appropriate electron acceptors are unavailable to the microflora (Lyngkilde and Christiansen, 1992; Bosma *et al*., 1996).

In the natural environment, the rate of degradation can be depended on physical, chemical and biological factors which may differ among ecosystems

(Melcer and Bridle, 1985). Alexander (1994) reported that for a microbial transformation to occur, a number of conditions must be satisfied (Lappin *et al*., 1985; Duetz *et al*., 1994).

2.7.1. Phenol-degrading microorganisms

Phenols are metabolized by microorganisms from a variety of different genera and species (Table 2.4). Bacteria, fungi, yeast and algae have been reported to be capable of degrading phenol. As shown in Table 2.4, *Pseudomonas putida* has been extensively investigated and has been reported to be capable of high rates of phenol degradation (Hutchinson and Robinson, 1988). According to Whiteley *et al*. (2001) isolates that were able to utilize phenol as a sole carbon source predominantly belonged to *P. pseudoalcaligenes*. The earlier reports on the decomposition of phenolic compounds by yeasts were by strains belonging to the genera *Oospora, Saccharomyces, Candida, Debaryomyces* and *Trichosporon cutaneum* (Neujahr and Varga, 1970; Neujahr *et al*., 1974). Among the yeast strains, *Candida tropicalis* has been the most studied and able to degrade phenol, phenol derivatives and aliphatic compounds at a relatively high concentration (Chang *et al*., 1998; Ruiz-Ordaz *et al*., 2000). Mutant strain of *Comamonas testosteroni* E23 has been regarded as the best phenol degrader of all phenol degrading strains reported up to date (Yap *et al.*, 1999).

Table 2.4 Phenol-degrading microorganisms.

Microorganisms	**Performance** (mg/l-h)	**References**
A. Bacteria		
Acinetobacter sp.	36-38	Hao *et al*., 2002
	16.7	Hao *et al*., 2002
- *A.* sp. W-17	4.2	Beshay *et al*., 2002
	6.9-12.2	Beshay *et al*., 2002
	4.2	Abd-EL-Haleem *et al*., 2003
	20-33	Abd-EL-Haleem *et al*.,2003
- *A. calcoaceticus*	7.6-25	Nakamura and Sawada, 2000
- *A. johnsonii*	11.8	Heilbuth *et al*., 2003
Achromobacter sp.E1	0.5	Watanabe *et al*., 1996
Alcaligenes faecalis	4.7-7.4	Bastos *et al*., 2000b
- *A.* sp. E2	0.4	Watanabe *et al*., 1996
-*A.* sp. R5	0.8	Watanabe *et al*., 1996
-*A.* strain P5	0.1-0.2	Baek *et al*., 2001
Arthrobacter sp.	62.5	Kar *et al*., 1996
Azoarcus sp.	1.8	Shinoda *et al*., 2000
Aureobasidium pullulans FE13	18.4	Santos *et al*., 2009
B. thermoleovorans-A2	7.8-19.6	Mutzel *et al*., 1996
Pseudomonas sp.	14-28	Kang and Park, 1997

Table 2.4 Phenol-degrading microorganisms (continued).

Microorganisms	**Performance** (mg/l-h)	**References**
- *P. aeruginosa* MTCC 4996	8	Kotresha and Vidyasagar, 2008
- *P. pictorum*-NICM-2077	20-46	Sheeja and Murugesan, 2002
- *P. putida* ATCC 11172	17	Loh and Liu, 2001;
	30-40	Mordocco *et al*., 1999
	250-297	Hannaford and Kuek, 1999
- *P. putida* – ATCC 12633	152-238	Hughes and Cooper, 1996
- *P. putida* – ATCC 17484	4.2	Gonzalez *et al.* 2001a
	3.5	Gonzalez *et al.* 2001a
	14	Tarighian *et al.* 2001
- *P. putida* – ATCC 21812	70-58	Daraktchiev *et al*, 1996
- *P. putida* – ATCC 49451	8-22	Wang and Loh, 1999
- *P. putida* F1-ATCC 700007	3-8	Abuhamed *et al*., 2003
	10-18	Abuhamed *et al*., 2004
- *P. putida* F1	0.5	Reardon *et al*., 2000
	0.8-0.9	Reardon *et al*., 2000
	1	Reardon *et al*., 2002
- *P. putida* F1	1-12	Chung *et al*., 2003
- *P. putida* BH	34-63	Soda *et al*., 1998

Table 2.4 Phenol-degrading microorganisms (continued).

Microorganisms	**Performance** (mg/l-h)	**References**
- *P. putida* BH-(ps10-45)(GEM)	40-91	Soda *et al*., 1998
- *P. putida* – CCRC14365	1-20	Chung *et al*., 2003
- *P. putida* – DSM 548	2	Monteiro *et al.*, 2000
- *P. putida* – MTCC 1194	4-9	Bandhyopadhyay *et al*., 1998
	5-10	Bandhyopadhyay *et al*., 2001
	3-11	Mahadevaswamy *et al*., 2004
-*P. putida* Q5	3	Kotturi *et al*., 1991
-*P. stutzeri*strain SPC2	38-48	Ahamad and Kunhi, 1996
-*P. testosteroni*-CPW301	<1	Kim *et al*., 2002
Ralstonia eutropha-ATCC 17697	50	Léonard *et al*., 1999
Rhodococcus sp. DCB-p0610	18	Pai *et al*., 1995
	86	Pai *et al*., 1995
- *R. erythropolis*-UPV-1	14-27	Prieto *et al*., 2002
	20	Prieto *et al*., 2002

Table 2.4 Phenol-degrading microorganisms (continued).

Microorganisms	**Performance** (mg/l-h)	**References**
- *R. erythropolis*-		
UPV-1	3-13	Hidalgo *et al*., 2002
Mixed bacterial cultures		
Mixed bacteria	0.8-2	Ha *et al*., 2000
Arthrobacter sp +, *B. cereus*, *C. Freundii*, *M. agilis*, *P. putida* b. B	7-14	Kanekar *et al.*, 1999
Bacteria + *E. coli*- ATCC 33456	<1	Chirwa and Wang, 2000
Clostridium ghonii, *C. hastiforme*, - *C. glycolicum*)	<1	Létourneau *et al*., 1995
P. putida F1 + *B.* strain JS150	2-3	Rogers and Reardon, 2000;
	1	Reardon *et al*., 2002
Coprinus sp.	0.8	Guiraud *et al*., 1999
-*C. cinereus*	0.8	Guiraud *et al*., 1999

Table 2.4 Phenol-degrading microorganisms (continued).

Microorganisms	**Performance** (mg/l-h)	**References**
Graphium LE6, LE11,LA1, LE9, LA5,FIB4,AE2	4	Santos and Linardi, 2004
Geotrichum-Candidum	<1-3	Garcia *et al*., 1997, 2000
Penicillium AF2, AF4,-FIB9	<1-4	Santos and Linardi, 2004
Pleurotus ostreatus	6-13	Fountoulakis *et al*., 2002
Phanerochaete-Chrysosporium	8	Garcia *et al*., 2000
C. Yeast		
Candida tropicalis		
- *C. tropicalis*	7-29	Bastos *et al*., 2000a
	99-191	Ruiz-Ordaz *et al*., 2001
-*C. tropicalis*-Ct2	157	Komarkova *et al*., 2003
-*C. tropicalis* – NCYC 1503	0.9-10	Chen *et al*., 2002
	6-7	Chen *et al*., 2002
-*C. tropicalis*	30	Yan *et al*., 2005

Table 2.4 Phenol-degrading microorganisms (continued).

Microorganisms	Performance (mg/l-h)	References
-*C. tropicalis* NCIM 3556	41.7	Varma and Gaikwad, 2008
Rhodotorula-glutinis ATCC 28052	26	Katayama-Hirayama *et al*., 1994
Trichosporon-Cutaneum R57 (mutant)	50-63	Alexieva *et al*., 2004
D. Alga		
Ochromonas danica	24	Semple and Cain, 1995
	12	Semple and Cain, 1995

Source: Modified from Mohd Tuah, 2006

2.8 Mechanism of phenol biodegradation

Generally aromatic compounds are broken down by natural bacteria. However, polycyclic aromatic compounds are more recalcitrant. Derivatisation of aromatic nuclei with various substituents particularly with halogens makes them more

recalcitrant. 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 monoxygenases and dioxygenases. The critical step in the metabolism of aromatic compounds is the destruction of the resonance structure by hydroxylation and fission of the benzoid ring which is achieved by dioxygenase-catalysed reactions in the aerobic systems. Based on the substrate that is attacked by the ring cleaving enzyme dioxygenase, the aromatic metabolism can be grouped as catechol pathway, gentisate pathway, and proto catechaute pathway. In all these pathways, the ring activation by the introduction of hydroxyl groups is followed by the enzymatic ring cleavage. The ring fission products, then undergoes transformations leading to the general metabolic pathways of the organisms. Most of the aromatic catabolic pathways converge at catechol. Catechols are formed as intermediates from a vast range of substituted and nonsubstituted mono and poly aromatic compounds. Aerobically, phenol is also first converted to catechol, and subsequently, the catechol is degraded via *ortho*- or *meta*- fission to intermediates of central metabolism. The initial ring fission is catalysed by an *ortho* cleaving enzyme, catechol 1, 2 dioxygenase or by a *meta* cleaving enzyme catechol 2,3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydro cis muconic semi aldehyde for the latter (Gurujeyalakshmi and Oriel, 1988).

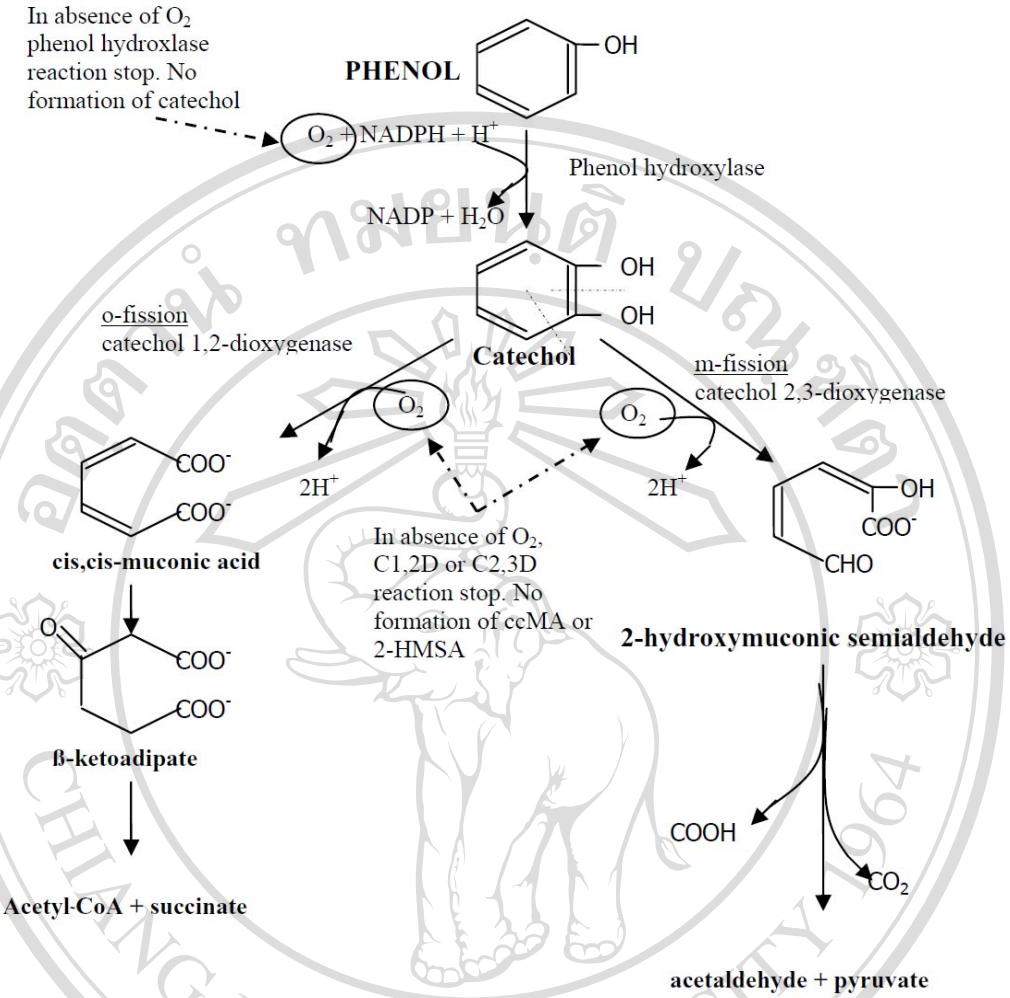


Figure 2.1 The main pathways of phenol degradation under aerobic condition (*ortho-* and *meta* fission of the benzene ring). (adapted from Krug *et al*., 1985)

2.9 Phenol-degrading *Candida tropicalis*

An investigation on the origin of the phenol-degrading *Candida tropicalis* was attempted previously, as shown in Table 2.5.

Yeasts are widely distributed in nature and have extremely diverse metabolic capabilities and can utilize a wide range of nutrients under a variable of environmental conditions (Tornai-Lehoczki *et al*., 2003). Among the yeast species, *Candida tropicalis* utilized a very large variety of carbon sources including many sugars, disaccharides, alkanes, alkane derivatives, fatty acids and phenols (Kawachi *et al*., 1997). Other industrial importance of *C. tropicalis* are production of xylitol (Azuma *et al*., 2000; Lima *et al*., 2003), crude oil-utilizers (Murzakov *et al*., 2003), and production of microbial protein and fodder yeast (Stanton and Dasilva, 1978).

Table 2.5 Source of origin of phenol degrading *Candida tropicalis.*

Candida tropicalis	**Source of origin**	**Reference**
C. tropicalis	activated sludge	Yan *et al*., 2005
C. tropicalis-YMEC14	olive mill wastewater	Ettayebi *et al*., 2003
C. tropicalis ct2	activated sludge of an industrial-wastewater treatment plant	Komarkova *et al*., 2003 Vojta *et al*., 2002 Bryndová, 2002
C. tropicalis-NCYC 1503	NA	Chen *et al*., 2002
C. tropicalis	soil from pristine Amazon-rain forest	Bastos *et al*., 2000
C. tropicalis	NA	Klein *et al*., 1979 Stephenson, 1990

Table 2.5 Source of origin of phenol degrading *Candida tropicalis* (continued).

Candida tropicalis	**Source of origin**	**Reference**
C. tropicalis CHP4	phenol-bearing industrial-wastes	Kumaran, 1980
C. tropicalis H15	NA	Krug *et al*., 1985 Krug and Straube, 1986
C. tropicalis 708	NA	Shimizu, 1973

NA = not available

As shown in Table 2.5, *C. tropicalis* capable of degrading phenol was found both in contaminated and pristine ecosystems.

2.10 Aerobic biodegradation of phenol

Microorganisms grown on phenol have been isolated (Hutchinson and Robinson, 1988). Microorganisms that can degrade phenol were isolated as early as 1908 (Evans, 1947). Bacteria play a major role in the degradation of phenol in the ecosystem; in soil (Hickman and Novak, 1989), sediments (Shimp and Young, 1987) and water (Howard, 1989). Despite being toxic, phenol can be utilized by microbes as carbon and energy sources (van Schie and Young, 2000). The number of bacteria capable of utilizing phenol is usually a small percentage of the total population present in, for example, a soil sample (Hickman and Novak, 1989).

In the last 20 years, studies have been performed on both aerobic (Oltmanns *et al*.,1989) and anaerobic (Knoll and Winter, 1987) treatment of aromatic pollutants by using pure microorganisms or pure culture. Aerobic processes of biological treatment are generally preferred to degrade phenolic compounds (Fedorak *et al.,* 1984) due to the low costs associated with this option, and as well as to the possibility of their complete mineralization (Collins and Daugulis, 1997). Studies on phenol toxicity to bacteria in phenol-contaminated sites have shown that bacteria can adapt to ambient phenol concentrations, but increasing phenol concentrations appear to decrease the overall biodegradation (Dean-Ross, 1989; Dean-Ross and Rahimi, 1995).

2.11 Phenol inhibitory levels for phenol degradation by microorganisms

Substrate inhibition is characteristic of toxic substrate metabolism (Santos and Linardi, 2004). The toxicity of phenol at high concentrations level could inhibit the related metabolism of degradation resulting in a lower efficiency by free cells (Chen *et al*., 2002). The observed phenol inhibitory level reported by previous researchers is shown in Table 2.6.

Table 2.6 Phenol inhibitory levels for phenol degradation by microorganisms.

Microorganism	**Observed phenol-inhibitory level** (mg/L)	**Reference**
*C. tropicalis, Trichosporon-cutaneum*and *Dabaromyces-subglobosus*(mixed culture)	300	Chai *et al*., 2004
NA	300	Yoong *et al*., 2004
Ralstonia eutropha 335 ATCC 17697	282	Léonard *et al*., 1999
P. putida Q5	<25-120	Onsyko *et al*., 2002
Comamonas testosteroni P15	117	Yap *et al*., 1999
Comamonas testosteroni E23	235	Yap *et al*., 1999
P. putida CCRC 14365	80	Chung *et al*., 2003
P. putida ATCC 700007	50	Abuhamed *et al*., 2003
P. putida ATCC 49451	50	Wang and Loh, 1999
Halophilic bacteria CA00	50	Peyton *et al*., 2002
P. putida NRRL-ß-14875	40	Seker *et al*., 1997
*Achromobacter*sp. E1	40	Watanabe *et al*., 1996
Alcaligenes sp. E2	30	Watanabe *et al*., 1996
Alcaligenes R5	30	Watanabe *et al*., 1996
P. putida DSM 548	25	Monteiro *et al*., 2000
P. putida MTCC 1194	25	Mahadevaswamy *et al*., 2004

Source: modified from Mohd Tuah, 2006

2.12 Factor affecting phenol degradation

2.12.1 Effect of temperature

Temperature might have an equivalent or larger role, than nutrient availability, in degrading organic pollutants (Margesin and Schinner, 1997). Most laboratory studies on pollutants degradation have been carried out at temperatures (mainly at 30 ºC) higher than those found in nature (van Schie and Young, 1998; Loh and Yu, 2000; Annadurai *et al.*, 2002). The effect of temperature on pollutants degradation has been investigated for oil (Margesin and Schinner, 1997), toluene, benzoate (Chablain *et al.,* 1997), chlorophenols (Melin *et al*., 1998; Cort and Bielefeldt, 2000) and nonylphenol polyethoxylate (Manzano *et al.,* 1999). However, the effect of a wide range of temperatures on phenol degradation by free cells of bacterial strains has not been studied extensively (Paraskevi and Euripides, 2005).

2.12.2 Effect of pH

Most natural environments *(i. e. ,* soils) have values of pH between 5.0 and 9.0; consequently, this range is optimal for microbial enhanced biodegradation of waste contamination. This pH range is maintained by a natural buffering capacity that exists in most fertile native soils due to the presence of carbonates and other minerals. However, this buffering capacity can be depleted over time as a result of acidic byproducts of degradation. Although microbes can adapt to a broader range of pH values, there typically is an accompanying decrease in growth/metabolic rates (Brockt

et al., 1984). Bacterial strains exhibited an optimal pH at approximately neutral for phenol degradation as reported at pH 6.8 for *Klebsiella oxytoca* (Shawabken *et al.,* 2007) and at pH 7 for *Pseudomonas* (Bandhyopadhyay *et al*., 1998).

2.12.3 Effect of secondary carbon source

The presence of simple carbon sources like organic acids and sugars, which are preferentially utilized by microorganisms, and unless the simpler carbon sources are completely depleted, the toxic aromatic compounds are not degraded (Collier *et al*., 1996). It has been reported that degradation of aromatic compounds is repressed by glucose as well as by organic acids (Schleissner *et al*., 1994; Muller *et al*., 1996; McFall *et al.*, 1997). Such preferential utilization of simple carbon sources represses degradation of recalcitrant compounds in nature (referred to as carbon catabolite repression). Attempts have been made to overcome this repression by generating mutants defective in glucose utilization which will mineralize complex carbon sources like naphthalene efficiently even in the presence of glucose (Samanta *et al*., 2001).

The addition of conventional carbon sources, such as glucose, yeast extract, and sodium glutamate has been considered a promising method to increase cells tolerance towards substrate inhibition (Wang and Loh, 2000). The result of this addition is improved phenol degradation, which can be attributed to the attenuation of phenol toxicity and the buildup of increased cell mass (Lob and Tar, 2000). Additional methods, which have been proposed to overcome substrate inhibition,

include genetically modified microorganisms (Soda *et al*., 1998), immobilization of cells (Santos *et al*., 2001; Loh *et al*., 2000) and gradual adaptation of cells to higher phenol concentrations (Guieyesse *et al.*, 2001).

For substrates such as organic acids, Babel *et al.* (1993) postulated that carbon sources in the environment may often be used simultaneously, although this mixotrophic behavior most likely predominates when only low (often limiting) carbon substrate concentrations are encountered. The repression of the catabolism of less favorable substrates by other carbon sources (often referred to as catabolite repression) has been extensively described for both enteric bacteria (Botsford and Harman, 1992) and gram-positive bacteria (Saier *et al*., 1996).

2.12.4 Effect of metal ions

In sites co-contaminated with metals and organic compounds, metal toxicity inhibits the activity of organic degrading microorganisms, impacting both their physiology and ecology, thus reducing the rate of biodegradation of the organic compounds (Roane *et al*., 2001; Maslin and Maier, 2000). Most commonly, metal inhibition of biodegradation has been related to the total metal concentration in a system. The concentration of the most bioavailable form (*i.e*., species) of the metal (commonly held to be the free, ionic, solution-phase metal species) is likely a better indicator of the extent to which a metal will inhibit biodegradation (Todd and Douglas). The inhibitory effect of copper was shown to be stronger than that of Zinc on phenol consumption by *Acinetobacter calcoaceticus* AH strain (Nakamura and

Sawada, 2000). Metal ions such as Fe, Cu, Zn and Mn at low concentrations stimulated and enhanced the rate of phenol degradation by *P. aeruginosa* MTCC 4996 (Kotresha and Vidyasagar, 2008)

2.13 Cells immobilization

2.13.1 Immobilization techniques

Immobilized cells have been defined as cells that are entrapped within or associated with an insoluble matrix (Mattiasson, 1983).

Techniques for the immobilization of microbial cells can be classified into three categories analogous to the immobilization of enzymes, that is, carrier-binding, cross-linking, and entrapping methods. Among these methods, the entrapping method has been most extensively studied (Chibata and Tosa, 1981).

2.13.1.1 Carrier-Binding Method

The carrier-binding method is based on direct binding of microbial cells to water-insoluble carriers via physical adsorption, ionic, and/or covalent bonds. As carriers, water-insoluble polysaccharides (cellulose derivatives and Sephadex), proteins (gelatin and albumin), synthetic polymers (ion-exchange resins and polyvinylchloride), inorganic materials (bricks and sand), and metal oxides (zirconium oxide and titanium oxide) are used (Chibata and Tosa, 1981).

However, this method is considered not to be advantageous, because enzymes and cells may leak out from the carrier because of autolysis of the cells during the enzyme reaction.

2.13.1.2 Cross-Linking Method

Microbial cells can be immobilized by cross-linking each other with bi or multifunctional reagents such as glutaraldehyde, toluendiisocyanate, or diazotized diamine. Although papers on this method are still few, there is a possibility that suitable cross-linking reagents for immobilization of cells will be found in the future (Chibata and Tosa, 1981).

2.13.1.3 Entrapping Method

Until now, a method of direct entrapping microbial cells into polymer matrices has been most extensively studied for immobilization of cells. In this method, the matrices are employed: (a) agar (Juaxrez-Ramixrez *et al*, 2001; Karigar *et al*., 2006; Zaki *et al*., 2008), (b) agarose (Zaki *et al*., 2008), (c) alginate (Santos *et al*., 2001; Karigar *et al*., 2006; Zaki *et al*., 2008), (d) carrageenan (Carmichael *et al*., 1986), (e) polyacrylamide (Chen *et al*., 2002).

2.13.2 Advantages and disadvantages

Immobilization allows higher biomass concentration, minimization of inhibition, resistance to chemical environments and column operations. Immobilization may also improve biomass performance, increase mechanical strength and facilitate separation of biomass from pollutant-bearing solution. Although numbers of advantages of using immobilized microorganisms, diffusion limitations are main disadvantages of the process. When biomass is immobilized the number of binding sites easily accessible to pollutant in solution is greatly reduced since the majority of sites will lie within the bead. So a good support material used for immobilization should be rigid, chemically inert and cheap. In addition, it should bind cells firmly, should have high loading capacity and should have a loose structure for overcoming diffusion limitations. (Gemeiner, 1992; Shuler and Kargi, 1992; Lee *et al*., 1994)

Immobilization has been shown to be an effective treatment in the biodegradation of phenol. *Pseudomonas putida* cells immobilized in polysulphone hollow fibre membranes were successful in degrading phenol at concentrations in excess of 500 mg/l (Loh *et al*., 2000). The potential of immobilized cells of *T. cutaneum* R57 is to degrade phenol with concentrations up to 1 g/l (Godjevargov *et al*., 1998). Continuous degradation of phenol from 100 mg/l to concentration as low as 2.5 mg/l with immobilized *P. putida* was reported (Mordocco *et al*., 1999). The immobilized cells of *Acinetobacter* sp. could tolerate a high phenol level and protected the bacteria against changes in temperature and pH (Wang *et al*., 2007). *C.*

tropicalis immobilized in agar was able to degrade phenol up 1560 mg/l in a fluidized bed reactor (Juaxrez-Ramixrez *et al.*, 2001). *Aureobasidium pullulans* FE13 immobilized in alginate (1,700 mg/l) (Santos *et al*., 2008). *Arthrobacter citreus* immobilized in alginate or agar (2,070 mg/l) (Karigar *et al*., 2006). The highest phenol degradation rate of immobilized *Nocardioides* sp. NSP41 in alginate was 21.0 mg /l-h at 400 mg/l phenol (Cho *et al*., 2000). Degradation rate of free and immobilized cells (in alginate) of *Aureobasidium pullulans* FE13 were 18.35 mg/l-h and 20.45 mg/l-h, respectively at 1505 mg/l phenol in batch cultures (Santos *et al*., 2008).

2.14 Repeated batch mode

Repeated batch cultivation is a well-known method for enhancing the productivity of microbial cultures (Radmann *et al.*, 2007). In repeated batch cultivation, the batch reactor is initially filled with the inoculum together with the cultivation medium and incubated under specific conditions. After a certain period, a specific volume of the culture is removed and replaced with an equal amount of fresh medium. Consequently a part of cultivation medium is kept in reactor as starting inoculum (Yamane, 1995, Radmann *et al.*, 2007). Repeated batch culture provides an excellent condition for controling the nutrients feed rate to optimize the productivity (Giridhar and Srivastava, 2001). Furthermore, this method has operational advantages, such as avoiding variation in the inoculum and thus maintaining the microorganism at high growth rates (Fabregas *et al*., 1996). In general, cell immobilization is particularly feasible for repeated batch fermentation because of its

easy operation, convenient separation of cells from broth and high density of cells (Vijaikishore and Karanth, 1986; Bisping *et al*., 1990; Yamane, 1995).