Chapter 2
2. Review of Literature

Phenol (C₆H₅OH) is a monohydroxy derivative of benzene. It has a melting point of 41 °C and a boiling point of 182 °C. It is manufactured industrially by diazotization of aniline, hydrolysis of aryl halide and alkyl fusion of sulfonate. It is colorless and has a distinct and persistent odor. Phenol is quite toxic when present in high concentrations. It has been widely used as germicide. Other disinfectants have been rated in terms of 'Phenol coefficient' i.e. relative disinfecting power of any chemical with respect to phenol. Biological degradation of phenols is possible when they are present in low concentrations.

2.1 Sources of Phenol in the Environment

Phenolic compounds found in the environment originate both from natural and anthropogenic sources. Plants synthesize a wide variety of phenolic compounds ranging from simple catechols to complex polymers like lignins. These complex polymers must be microbiologically degraded for the recycle of carbon, where phenols are intermediates. Phenolics are also formed in the aerobic microbial degradation of many aromatic hydrocarbons such as napthalene, toluene, benzene and pesticides. Phenol is the basic raw material for the manufacture of various organic chemicals (Fig 2.1). A large fraction of its supply goes into the production of plastics (bakelite) and resins.
Fig 2.1 Use of Phenol in different industries
They are also used in preparing dyes, photographic developers and wood preservatives.

They are major toxic pollutants in the wastewater emanating from industries such as petrochemicals, plastics, petroleum refineries, coke and resins manufacturing industries. In these wastewaters phenol is a major compound of this group with varying quantities of other derivatives. Table 2.1 shows phenol concentration in a variety of industrial wastewaters.

2.2 Effects of Phenol

Phenol causes objectionable taste and odor in water. The medicinal taste of phenol is enhanced by chlorination, a common process in water treatment (1). Their effects, when it is present in a particular water body, are described here (2).

a. Domestic water supplies

The ingestion of a concentrated solution of phenol results in severe pain, renal irritation, shock and possible death. A total dose of 1.5 g may be fatal. It is unlikely, however, that such harmful concentrations of phenol will be consumed in drinking water because of adverse taste consideration (2).
b. Industrial water Supplies

Presence of phenol in water supplies is deleterious for many food and beverages industries and may cause obnoxious tastes and odors. It has been reported that pears packed in syrups made from chlorinated river waters had 'iodoform' tastes (2).

On the other side of the ledger, however, Pickett (3) reports that refineries discharge wastes containing upto 60 mg.L⁻¹ of phenol into Dominguez Channel in Los Angeles county without harmful effects and that a beneficial action occurs in that it retards the development of objectionable marine growths in the harbor. Similar discharges elsewhere might be highly objectionable (2).

c. Irrigation waters

Phenol in irrigation water is not considered to be deleterious to crops. In Germany, a municipal sewage containing 40-50 mg.L⁻¹ of phenol from a gas plant was used for irrigation without any damaging effect on the crops (2).

d. Stock and Wildlife Watering

It is reported that rats that drank water containing phenol from 15 to 1000 mg.L⁻¹ showed no deleterious physiological effects. In concentrations upto 5000 mg.L⁻¹, phenol did not interfere with
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<tr>
<th>Industry</th>
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<td>Coking Plant</td>
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<td>Weak ammonia liquor without dephenolization</td>
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<td>Weak ammonia liquor after dephenolozation</td>
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<td>Wash oil still wastes</td>
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<td>Oil refineries</td>
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<td>Sour water</td>
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<td>General wastewater</td>
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<td>APF separator effluent</td>
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<td>Petrochemicals</td>
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<td>Benzene refineries</td>
<td>210</td>
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<td>Tar distillation</td>
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<td>Nitrogen works</td>
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<td>Orlon manufacturing</td>
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<td>Plastics Factory</td>
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<td>Fibre glass Manufacturing</td>
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- Adapted from Patterson (4)
digestion, adsorption or other metabolic functions. Above 7000 mg.L⁻¹, growth was stunted and many young died at birth (2).

e. Fish and Other Aquatic Life

Phenolic compounds may effect fish in two ways: first, by direct toxic action and second, by imparting a taste to the fish flesh. The lethal concentrations vary widely not only because of the common variables such as species, temperature, time of contact, dissolved oxygen and mineral quality of water but also because of synergistic and antagonistic effects of other substances in the water. Many phenolic compounds are more toxic than pure phenol and consequently mixed phenolic compounds may have a lower lethal limit than phenol itself (2).

It appears to be less toxic towards fish-food organisms and other lower aquatic life than towards fish. It is less toxic than cresols, but more so than catechol for algae. The toxic effect of phenol towards algae is essentially attributable to its undissociated molecule and to the tendency to form compounds with albumin of the protoplasm (2).

On the basis of the above discussions, it appears that the following concentrations of phenol will not interfere with the designated beneficial uses:
Domestic water supply: 0.001 mg.L⁻¹
Irrigation: 50.0 mg.L⁻¹
Stock watering: 1000.0 mg.L⁻¹
Fish and aquatic life: 0.2 mg.L⁻¹

As per Bureau of Indian standards (BIS) acceptable limits of phenol in treated wastewaters for different water receiving bodies are as follows:

- Surface waters: 1.0 mg.L⁻¹
- Public Sewers: 5.0 mg.L⁻¹

2.3 Treatment Options

Various treatment options available for phenolic wastewaters are Physical, Physicochemical, Chemical, Enzymatic and Biological processes. Each of these treatment options are briefly discussed below.

2.3.1 Physical Processes

Physical processes used for phenol removal are solvent extraction and photo-oxidation using U.V. radiation. Solvent extraction procedure is practiced for the recovery of phenols from concentrated industrial wastewaters. The criteria for choosing the appropriate solvent for extraction are low water solubility, no emulsion formation with water and must be easily regenerable. Among many solvents, which include octane, mixtures of benzene with butylacetate and di-isopropyl, isobutylacetate
and isopropyl ether two are extensively used for dephenolising wastewaters (5). Solvent extraction process can typically reduce phenol concentrations from as high as 17,000 mg.L\(^{-1}\) to 920 mg.L\(^{-1}\) (6). Dephenolised wastewater will have to be treated by other suitable methods for further removal of phenol.

Kang et al. (7) reported that the photo-oxidation of wastewater using low pressure mercury lamp irradiation and H\(_2\)O\(_2\) as oxidant, is effective for removal of phenol at pH 5-8 and at 30 °C - 50 °C. Scheck and Frimmel (8) reported that phenol can be removed from water by oxidizing it with U.V radiation. The irradiation of photoreactor was done by low-pressure mercury lamp.

2.3.2 Physico- Chemical Processes

Adsorption method can be used for the removal of phenols from contaminated drinking water sources as well as for wastewaters that contain moderately low phenol concentrations. It is a separation process in which phenols are transferred from aqueous phase to the surface of adsorbent. Extensively used adsorbent is activated carbon. Commercially available activated carbons are derived from natural materials such as coconut shell, wood charcoal, which are carbonized at controlled conditions so as to have precise surface properties and high surface area. Other source materials, such as straw, used rubber tyres,
fertilizer waste slurry have also been tried in an attempt to produce low cost activated carbon (9,10).

Najam et al. (11) used powdered activated carbon (PAC) in upflow floc blanket reactor for the adsorption of 2,4,6 trichlorophenol (TCP). PAC adsorption capacity in floc blanket reactor decreased with decreasing influent trichlorophenol concentration.

Sorial et al. (12) studied the impact of molecular oxygen for adsorption behavior of a mixture of phenolic compounds on fixed bed GAC reactor.

Srivastava and Tyagi (9) prepared a cheap activated carbon from the waste slurry generated in fertilizers plants. The activated carbon was successfully tried for the removal of a variety of substituted phenols like chloro, nitro and trinitrophenols.

Streat et al. (10), used novel activated carbon prepared by carbonization and subsequent activation of straw and used rubber tyres as well as conventional activated carbon derived from coal, coconut shell and wood. The sorption kinetics of the straw and rubber tyres based carbon was identical to conventional activated carbons.

The activated carbon regeneration requires its burning at a temperature of 600 °C under controlled oxygen and moisture conditions, thus needing energy. About 5 to 10 % carbon is lost in regeneration. These two factors make the application of this process as cost intensive.
2.3.3 Chemical Treatment

Many oxidants like ozone, $\text{H}_2\text{O}_2$ and permanganate have been used for the chemical oxidation of phenols. It has been reported that ozonisation can result in structural modifications of pollutants, which make them amenable for biodegradation. A requirement of 7.5 Kg of ozone per Kg of phenol present, is reported for oil refineries wastewaters, if effluent quality of $0.2 \text{ mg.L}^{-1}$ is to be achieved (13, 14). Reaction between phenol and hydrogen peroxide is slow under non-catalyzed conditions. However, in the presence of Fe (II) ions, $\text{H}_2\text{O}_2$ oxidizes phenol to hydroquinone and catechol, which is further oxidized to quinones, carboxylic acid and finally to $\text{CO}_2$. Fenton’s reagent ($\text{H}_2\text{O}_2 = \text{Fe (II)}$ catalyst, pH 2-4) is reported to be the cheapest oxidation system as compared to ozone, chlorine dioxide and potassium permanganate. If the wastewater contains phosphates, catalytic action is reduced and the oxidation of phenols cannot be achieved.

Striolo et al. (15) utilized wet hydrogen peroxide oxidation process at 120 °C for treatment of aqueous organic wastewaters. The process was suitable for organic loads upto few grams per litre. The process is energy intensive as temperature of wastewater is to be brought to 120 °C.

Trapido et al. (16), studied the ozonation process of some phenols produced at different pH conditions. Results showed that in acidic and neutral media, ozonation of phenol is mainly a chemical reaction limited and in a basic media mass transfer is rate limited.
2.3.4 Enzymatic treatment

Enzymes are proteins that are synthesized by a living cell and act as catalysts for biological reactions. Enzymatic approach for the treatment of phenolic wastewaters is now attracting wide attention. It has been reported peroxidases can decolorise phenolic industrial effluents (17). Use of enzymes such as, tyrosinase and laccases are now being explored. These enzymes convert soluble phenolics to insoluble polyphenolic precipitates, which can be removed by filtration. Although enzymatic conversion of phenols to quinone is very rapid, nonenzymatic formation of oligomers and polyphenols leading to insoluble polyphenols is a very slow process and may require several hours. Chitin and chitosan which are available abundantly as shellfish waste, have been used for the removal of soluble oligomers and polymers (18).

The reduction order of substituted phenols is catechol > p- cresol > p chloro phenol > phenol > p-methoxy phenol. Reduction rate of phenols was also accelerated in the presence of chitosan. The mushroom tyrosinase enzyme is quite expensive and cannot be used for practical application. Immobilization of the enzyme by using amino groups in the enzyme on cation exchange resins has been reported for the repeated use of the enzyme for phenol oxidation (19).

Yimin et al. (20) have recently for the oxidation of phenols in the presence of mushroom tyrosinase, developed a model that includes a second order Michaelis-Menten equation with respect to the
concentration of phenol and hydrogen peroxide. Comparison between experimental data and calculated phenol concentrations proved that the model can be used under a variety of experimental conditions for prediction of phenol removal and the enzyme activity depletion are well predicted.

Advantages of enzymatic approach are the broad specificity of the enzymes, which enable them to react with a wide range of phenols, suitability for dilute wastewaters and less sensitivity to the operational upsets. Although the method appears to be feasible, presently used enzymes are costly. Thus field applications of the enzymatic methods depends on the availability of cheaper microbial source for the enzymes.

2.3.5 Biological Processes

The biological processes are always preferred over other treatment processes because complete mineralisation of organic carbon can be achieved with this system. Based on the terminal electron acceptor, biological system can be divided into different categories such as aerobic, iron reducing, sulfate reducing, nitrate reducing and methanogenic. Equations given below compare the free energies available, when selected electron acceptors are used (21). The maximum energy is available when oxygen is used as the terminal electron acceptor.

\[
O_2 + 2H_2 \rightarrow 2H_2O \quad \Delta G^0 = -237 \text{ KJ/H}_2
\]
The ability of bacteria to utilize aromatic hydrocarbons for growth was first demonstrated in 1908 by Stormer (22) who isolated *Bacillus hexacarbovorum* by virtue of its ability to grow on toluene and xylene. In 1913 Sohngen reported the utilization of benzene by microorganisms (23), and one year later Wagner isolated two organisms, *Bacterium benzoli a* and *b*, both of which were capable of growth on benzene, toluene and xylene (24). Gray and Thornton (25) demonstrated the ubiquitous distribution of soil bacteria capable of metabolizing aromatic compounds. These authors examined 245 soil samples and showed that 146 of the samples contained bacteria capable of oxidizing naphthalene, phenol or cresol. These initial observations paved the way for detailed studies on the degradation of aromatic compounds.

### 2.3.5.1 Aerobic Processes

Aerobic degradation of phenolics in the presence of O₂ has been extensively studied and reviewed. Aerobic metabolism can be divided into peripheral and central pathways (26,27). Initial goal in the aerobic degradation of aromatic compounds is to remove substituents from the ring and introduce hydroxyl functions. This facilitates dearomatisation
and subsequent ring cleavage. This achieved in peripheral pathways, which converts large variety of aromatic compounds to few central intermediates (Fig 2.2). The enzymes of peripheral pathways are often coded by genes on plasmids and are induced by the corresponding substrates. The usual prerequisite for the aromatic ring fission under aerobic conditions is the requirement of two (OH) groups that either ortho or para to each other. Molecular oxygen used for these reactions, which are catalysed by specific mono or dioxygenases. Dihydroxy aromatic compounds, which are thus formed are the central intermediates of aerobic metabolism and are generally ready to be dearomatised. In some cases, for example, in the degradation of thymol, orcinol, resorcinol and gallic acid, a third hydroxyl group must either be present or to be introduced before ring cleavage can occur.

Dearomatisation or the ring cleavage is carried out oxygenolytically by incorporating two atoms of oxygen in the presence of dioxygenases. There are two distinct modes of oxidative cleavage of the benzene nucleus. Cleavage of the bond between adjacent carbon atoms that carry hydroxyl group is known as "ortho" or "intra" cleavage, and the pathway by which the product of such cleavage is metabolized is known as ortho or \( \beta \)-ketoadipate pathway. Cleavage of the carbon-carbon bond in such compounds is by dioxygenases that are best designated by the name of the compound that is attacked and position of the carbon-carbon bond cleaved.
In the second mode of cleavage of the benzene nucleus, termed as *meta* pathway, attack occurs between two carbon atoms, only one of which carries a hydroxyl group, the other carbon atom being either unsubstituted or substituted with other than a hydroxyl group. In this case, hydroxyl group may be either *ortho* or *para* to one another, and the enzymes catalysing such cleavages are again usually designated by the position of the carbon bond attacked.

*Meta* pathways are regarded as being used for the degradation of wider range of compounds than the *ortho* pathway and may be attributed to the low specificity of both induction and function of several enzymes of pathways. Tricarboxyclic acid cycle (TCA) intermediates are end products of both ortho and meta pathways (Fig2.3).

### 2.3.5.2 Anaerobic Processes

Anaerobic ecosystems are created when oxygen consumption exceeds its supply; e.g., in soils with impeded drainage, stagnant water, municipal land fills, sewage treatment digestors, industrial plants that produce methane from organic waste, the alimentary tract of all animals and finally sediments of oceans and other natural water bodies. The metabolic fate of organic compounds and their mineralisation to CO₂ depends on the availability of light or of inorganic electron acceptors such as NO₃⁻, SO₄²⁻ or CO₂. Like aerobic process, anaerobic metabolism can
Fig 2.2 Peripheral pathways for aerobic metabolism of aromatic Compounds
Fig 2.3 Central pathways for catechol metabolism
also be divided into peripheral and central pathways (26,27). The essential step in anaerobic aromatic metabolism is the replacement of all the oxygen-dependent steps by an alternative set of novel reactions and the formation of different central intermediates (e.g., Benzoyl CoA) for breaking the aromaticity and cleaving the ring; notably, in anaerobic pathways, aromatic ring is reduced rather than oxidised.

Peripheral pathways convert the large variety of aromatic compounds into few central aromatic intermediates. The most common central intermediate in anaerobic aromatic metabolism is benzoyl-CoA (Fig 2.4). Of course, many other aromatic substrates and peripheral pathways are also used. Resorcinol and phloroglucinol are the central intermediates for other complex aromatic compounds (Fig 2.5). The common key enzyme for ring reduction in these metabolic routes is benzoyl-CoA reductase (dearomatising). The best studied organisms employing this pathway are *Rhodopseudomonas palustris* (28). The product of benzoyl-CoA reduction, cyclohex-1,5-diene-1-carboxyl-CoA, is oxidised so that 1 molecule produces 3 molecules of acetyl-CoA and 1 of CO$_2$ in a sequence of reactions that are analogous to $\beta$-oxidation. Central pathways for the conversion of benzoyl-CoA, phloroglucinol and resorcinol to acetyl-CoA are given in Figures 2.6 and 2.7.

Studies on aromatic degradation under different trophic conditions are briefly discussed below.
Fig 2.4 Peripheral Pathways transforming some aromatic compounds to Benzoyl CoA under anaerobic conditions
Fig 2.5 Peripheral pathways transforming some aromatic compounds to Phloroglucinol and Resorcinol under anaerobic conditions
Fig 2.6 The central benzoyl CoA pathway for anaerobic aromatic degradation
Fig 2.7 The central phloroglucinol/resorcinol pathways for anaerobic aromatic degradation
2.3.5.2.1 Photometabolism

Early attempts to elucidate the anaerobic breakdown of aromatic acids such as benzoate were based on a literal interpretation of Van Niel's (29) general scheme of photosynthesis in bacteria and plants. One of the products of light reaction was strong oxidant (OH) which, in plants, was converted into molecular oxygen; in bacteria this proposedly light induced "bound oxygen" was used to oxidise substrates. Proctor and Scher (30) surmised in 1960 that the light induced oxidant and molecular oxygen were equivalent and that the anaerobic photosynthetic metabolic pathway of benzoate was similar to the pathways of well known aerobic processes; however, Leadbetter and Hawk (31) and Dutton and Evans (32) could not reproduce the results of Proctor & Scher. They showed that phototrophic bacteria *Rhodopseudomonas palustris* cells, when grown photosynthetically on benzoate or 3 hydroxybenzoate, did not promote any respiratory response in the cells under aerobic conditions. Hence oxygen and any light-generated oxidant were not equivalent. However, under anaerobic conditions, light permitted benzoate utilisation proceeded effectively.

In 1968 Dutton and Evans (28) determined the key intermediates of anaerobic photosynthetic metabolism of benzoate in *Rhodopseudomonas palustris*. Five products incorporated the isotope: Cyclohex-1-ene-carboxylate, Cyclohexanecarboxylate, 2-hydroxycyclohexanecarboxylate, 2-oxocyclohexanecarboxylate and
pimelate. Interpreting these results, Evans and Fuchs (33) proposed that aromatic ring may become fully reduced with incorporation of six hydrogen equivalents to form cyclohexanecarboxylate. This was confirmed by Perotta and Harwood (34). The subsequent reactions, starting from fully saturated alicyclic acid, would be analogous to a classical β-oxidation of fatty acid Fig 2.6.

2.3.5.2.2 Iron Reducing

Ferric iron reducing microorganisms out-competes sulfate reducing bacteria and methanogens when ferric iron is present as amorphous ferric oxyhydroxide (35). There are very few reports on degradation of aromatic compounds by iron reducing microorganisms. It was only in 1990 Lovely and Lonergan (36) isolated a dissimilatory Fe (III) reducer, GS-15, which was the first microorganism known to couple the oxidation of aromatic compounds to the reduction of Fe (III). It is also the first example of the pure culture of any kind known to anaerobically oxidize an aromatic hydrocarbon, toluene (Fig 2.8). They described the degradation of p-cresol and phenol to CO₂ by the same isolate. Evidence of para-carboxylation was found, as 4-hydroxybenzoate was detected as a transient intermediate in the culture medium of GS-15. Methyl group oxidation of p-cresol and its subsequent conversion of p-hydroxy benzoate were also reported by these investigators. Thus these peripheral pathways lead to the formation of benzoyl CoA (Fig 2.6)
Fig 2.8 Pathway for the oxidation of toluene to benzoate coupled to Fe (III) reduction

2.3.5.2.3 Sulfate Reducing

Sulfate reducing bacteria couple the oxidation of organic compounds with water to the exergonic reduction of sulfate via sulfite to sulfide. Energy is derived mainly from electron transport phosphorylation during sulfite reduction. Sulfate reducers are mainly responsible for degradation of organic matter in anaerobic marine environments that contain approximately 27mM sulfate.

Under anaerobic conditions, most methoxylated mononuclear aromatic compounds are degraded by bacteria, with catechol being formed as an important intermediate (37). Gorny and Schink (37) on the basis of their experiment with sulfate-reducing bacterium desulfovibrio sp. strain Cat 2, described for the first time the enzymatic activities involved in the complete anaerobic oxidation of catechol and protocatechuic. Results demonstrated that all enzymes necessary for protocatechuate and benzoate degradation were induced during growth with catechol (Fig 2.9). Anaerobic oxidation of catechol was found to be dependent on the
Fig 2.9 Initial reactions of anaerobic catechol degradation by *Desulfo bacterium sp.*
presence of CO₂. Phenol was not degraded in cell suspensions grown on catechol. Degradation of benzoates coupled with sulfate reduction has been demonstrated in pure cultures (38) and in cocultures (39).

2.3.5.2.4 Methanogenic

Bacterial methanogenesis occurs on vast scale in anoxic non-marine environments and is mainly responsible for the degradation of organic matter in these ecosystems. In 1934 Travin and Buswell (40) provided the first decisive chemical evidence that the aromatic compounds were completely decomposed when incubated anaerobically with sewage sludge. Virtually all of the ring carbons of the aromatic substrates were accounted by CO₂, CH₄ and microbial cells. This pioneering quantitative study of the methanogenesis of organic compounds established the stochiometry of reaction as follows:

\[
C_nH_{a}O_b + (n - a/4 - b/2) H_2O = (n/2 - a/8 + b/4) CO_2 + (n/2 + a/8 - b/4) CH_4
\]

The fact that the phenomenon occurs in a mixed culture probably inhibited further progress for sometime; the doctrine of single substrate single organism had become entrenched. With the advent of radioactive tracers, Clark and Fina (41) showed that in a methanogenic culture adapted to metabolise benzoate, (ring-¹⁴ C), benzoate was completely degraded, and 50% of the radioactivity was recovered as CH₄.
cultures of methanogenic bacteria are able to use only few simple substrates for growth, e.g., acetate, formate, methanol and CO₂, where hydrogen is used as electron donor for CO₂ reduction. All methanogenic consortia must therefore rely on syntrophic associations with fermenters that degrade complex organic compounds into usable products for methanogens.

In fermentation, microorganisms derive their energy from substrate level phosphorylation reaction. Organic compounds serve as electron donors as well as acceptors. That is, inorganic electron acceptors such as nitrate, sulfate or carbon-dioxide are not involved. Patel et al. (42) and Tsai and Jones (43) were first to isolate species of Coprococcus and Streptococcus from rumen that fermented phloroglucinol via dihydrophloroglucinol to acetate. Schink and Pfennig (44) isolated five strains of a strictly anaerobic bacterium from marine mud with gallic acid, pyrogallol, phloroglucinol or 2,4,6 trihydroxybenzoate as substrates. These substrates were fermented stoichiometrically to 3 moles acetate and 1 mol CO₂.

Tschech and Schink (45) studied anaerobic fermentative degradation of resorcinol and α,β,γ resorcyclic acids in enrichment cultures. Resorcinol and β and γ-resorcylic acid were stoichiometrically converted to acetate and butyrate (Fig 2.7).

Krumholz and Bryant (46) isolated an anaerobic chemoorganotroph, Syntrophococcus succrotumans from the rumen, that cleaved methyl
ether linkages of substituted monobenzoids. Krumholz and Bryant (47) also isolated from the rumen a strictly anaerobic chemoorganotroph, *Eubacterium oxidoreducens* that degraded gallate, pyrogallol and phloroglucinol to acetate, butyrate and occasionally CO$_2$. It required either formate or hydrogen as electron donor to catabolize these aromatic substrates. A facultative anaerobe, *Enterobacter cloacae* fermentatively O-demethylated and dehydroxylated ferulate (3-CH$_3$O-4-OH-cinnamate) and reduced the side chain to phenylpropionate; the latter was metabolised further to lower fatty acids (48). Isotopic trapping experiments using $^{14}$C-labeled benzoate afforded cyclohexanecarboxylate, cyclohex-1-enecarboxylate, heptanoate, valerate, butyrate and acetate as fermentation products. These products were also identified during benzoate degradation by different consortia made up of multiple species.

Ferry and Wolfe (49) showed that the methanogenic fermentation of benzoate required the cooperation of several groups of bacteria and that the methanogens served only as terminal organism of a metabolic food chain. Sheridan *et al.* (50) and Dwyer *et al.* (51) observed a consortium consisting of three types of bacteria that were responsible for the conversion of phenol to CH$_4$ and CO$_2$. The following steps in the overall conversion of phenol to CH$_4$ were proposed by Sheridan *et al.*

\[
C_6H_6 + 5H_2O \rightarrow 3 \text{CH}_3\text{COOH} + 2\text{H}_2
\]

\[
3 \text{CH}_3\text{COOH} \rightarrow 3\text{CH}_4 + 3\text{CO}_2
\]
Degradation of phenolic compounds requires at least two microbial trophic groups. First group converts aromatic to acetyl CoA, which can be utilized by second group i.e. methanogens to form methane.

2.3.5.2.5 Nitrate Reducing

Nitrate respiration is used in the absence of oxygen by a few facultative heterotrophic anaerobic bacteria. Various organic compounds can serve as electron donors and nitrate is used by the organism as terminal electron acceptor leading to the formation of gaseous end products such as nitrogen and oxides of nitrogen. This process of denitrification is termed as 'anoxic' instead of anaerobic because principal biochemical pathways for energy release are not anaerobic, but only a modification of aerobic pathway. Many phenolics are biodegraded under anoxic condition (52). Aromatic ring cleavage however follows a pathway similar to anaerobic process because of the lack of oxygen in the environment (53).

In the denitrification process, nitrate is reduced to molecular nitrogen via several intermediate stages. Focht and Chang (54) proposed the following path:

\[
2H_2 + 0.5 CO_2 \rightarrow 0.5 CH_4 + H_2O
\]

\[
C_6H_6O + 4H_2O \rightarrow 3.5 CH_4 + 2.5 CO_2
\]
Denitrification is a process that takes place under anaerobic conditions, where the links of the above mentioned reaction chain act as electron acceptors. Each step in the denitrification process is catalyzed by a separate enzyme system. Nitrate is used preferentially over nitrite, even though both nitrate and nitrate reductases are present.

Many facultative anaerobes are capable of carrying on denitrification, which include **Bacillus**, **Pseudomonas**, **Alcaligenes**, **Micrococcus**, **Spirillum**, **Morcella**, **Hyphomicrobium**, **Xanatmonas**, **Glucanobacter** etc. Only few phenolytic denitrifiers belonging to genus **Thaurea**, **Azoracus**, and **Magnetospirillum** have been reported (55,56,57), which can degrade phenol as well as several other aromatic compounds such as **m-cresol**, **salicylic acid** and **m-hydroxy benzoate** under denitrifying conditions (58,59). Most of these aromatics are converted to benzoyl CoA during the degradation. Phenol degradation by these cultures are **CO₂** dependent (60). They showed that initial reactions in the degradation pathway in denitrifying cultures, phenyl phosphate and **p-hydroxy benzoyl CoA** are formed. **p-hydroxy benzoyl CoA** was reductively dehydroxylated to form central intermediate benzoyl-CoA. Benzoyl-CoA was subsequently degraded as shown in Fig 2.10.
Fig 2.10 Phenol degradation by denitrifying *Pseudomonas sp.*

(1) Phenol kinase activity (2) Phenylphosphate carboxylase activity; phenol carboxylase system consists of activities (1) and (2); (3) 4-hydroxybenzoate-CoA ligase (4) 4-hydroxybenzoyl-CoA reductase.
2.4 Applications of the Biological Processes

2.4.1 Aerobic wastewater treatment

Aerobic biological processes are being extensively used for the treatment of phenolic wastewaters.

Capestany et al. (61) reported that an activated sludge plant fed with phenol at 1000 mg.L\(^{-1}\) and operated at hydraulic retention time of 24hr produced an effluent of 0.5 mg.L\(^{-1}\) phenol concentration. Resulting in more than 99% treatment efficiency.

Rozich et al. (62,63) determined microbial kinetic constants for phenol degradation using batch as well as continuously fed reactors with partial sludge recycle. Among the various kinetic models (64), Haldane equation gave satisfactory predictions with respect to biomass growth and effluent substrate concentration.

A completely mixed activated sludge (CMAC) was operated at laboratory and pilot scale for treatment of synthetic and live wastewaters (65). Influent phenol concentration of 1000 mg.L\(^{-1}\) in synthetic phenolic wastewater at 10 hr HRT was used for kinetic study. COD removal efficiency was 94 to 96 %.

Tyagi et al. (66) assessed the feasibility of modified rotating biological contractor with polyurethane foam attached to the disks as porous support media to biodegrade petroleum refinery wastewater. Ammonia nitrogen and phenol removal was above 99 and 85 % respectively.
Kumaran (67) studied the use of specialized microbes *Candida tropicalis*, *Acinetobacter calcoaceticus*, and *Pseudomonas putida* for phenolic waste management. Results showed that specialized microbes can be used as the starter culture seed in laboratory treatability studies as well as full scale wastewater treatment plants.

Dikshitulu *et al.* (68) studied the competition between two microbial populations for phenol in a sequential batch reactors. A mathematical model describing this system was developed and tested experimentally. It is based on the specific growth rate expressions derived from pure culture batch experiments. The species employed was *Pseudomonas putida* and *Pseudomonas resinovorans*.

Chakravarty and Maiti (69) used sequential batch reactors (SBR) to develop an activated sludge enrichment culture capable of degrading phenolic wastewater containing nearly 800 mg.L⁻¹ of phenol and 750 mg.L⁻¹ of ammonia. 99% removal in terms of chemical oxygen demand was observed.

Kibret *et al.* (70) studied characterization of a phenol degrading mixed population by enzyme assay. The metabolic response of a mixed population to stepwise increase in phenol concentration was followed with the assay of pyrocatechase activity. Pyrocatachase activity was dependent on the concentration of phenol in the aeration tank. From pyrocatachase activity assay it was possible to demonstrate the metabolic response of the activated sludge to changes in phenol.
concentration. The accumulation of phenol and decrease in pyrocatachase activity are considered as the stage when the system no longer copes with increase in phenol loading.

Although these aerobic treatment processes are highly efficient, they are limited by the availability of molecular oxygen, which has to be replenished using aeration system.

2.4.2 Anaerobic Wastewater treatment leading to methanogenesis

Traditionally anaerobic processes were used for the stabilization of sludge generated from aerobic treatment plants and concentrated animal waste. Basic insight to anaerobic process and the introduction of novel retained biomass reactors have led to the application of this process to domestic as well as industrial wastewaters, especially because of its advantages over aerobic treatment processes. They include low sludge production, no energy intensive aeration requirement and the production of high BTU methane gas as one of the products.

Wang et al. (71) studied the biodegradability of phenol and substituted phenols in batch phenol enriched methanogenic cultures. Phenol, at concentrations up to 1400 mg.L\(^{-1}\) was completely degraded to methane and carbon dioxide in 350 hr incubation.

Khan et al. (72) studied the removal of phenol from a synthetic nutrient-supplemented wastewater using a three stage, anaerobic-activated
carbon filter with intermediate effluent recycle. They performed experiments with empty bed contact times of 9.3 and 18.6 hr. Removal efficiency ranging from 92.5 to 100% were observed at phenol loading rates from 0.26 to 2.58 Kg phenol per cubic meter per day.

Suidan et al. (73,74,75) studied phenol, o-cresol and catechol treatment in multistage, anaerobic activated carbon columns with an intermediate effluent recycle. The synthetic waste medium was qualitatively similar to that reported by Khan et al (72). Phenol and catechol were degraded with methane production, whereas o-cresol was removed but no degradation to methane was found. This indicated that o-cresol was adsorbed rather than degraded.

Norrman (76) treated Kraft mill, black liquor evaporator condensate in fixed bed, expanded bed and fluidized bed anaerobic film reactors. More than 80% COD reduction was achieved at COD loading of 2Kg COD per cubic meter per day.

Wang et al. (77) studied the organic removal efficiency of an expanded bed anaerobic reactor using granular activated carbon (GAC) as a biological attachment, medium was high when subjected to a wide range of feed phenol concentrations. Granular activated carbon expanded-bed reactor achieved nearly 100% removal of phenol when the surface loading ranged from 0.028 to 0.23 mg.cm^{-2}.day^{-1} COD.

Craik et al. (78) used continuous feed recycle bio-reactor for degradation of phenol by bacteria supported on a bed of GAC and granular biomass.
GAC reduced the toxicity of phenol to the microorganisms. The specific phenol degrading activity of biomass supported on GAC was inferior to that of granular biomass and suspended biomass. Nakhala and Suidan (79) studied expanded bed anaerobic GAC reactor, operating with GAC replacement for treating toxic wastewaters. GAC adsorbed the toxic pollutants as well as provided surface for microbial attachment. The problem with using GAC was its exhaustible adsorptive capacity, thus demanding replacement of exhausted GAC with fresh one for long term operations. Fang et al. (80) observed that phenol in wastewater was effectively degraded in upflow anaerobic sludge blanket (UASB) reactor at loading rate of 6-gm COD.L⁻¹.day⁻¹. With 1:1 recycle ratio, over 97% phenol was removed at 37°C, pH 6.8-7.5 and an HRT of 12 hr for phenol concentrations up to 1260 mg.L⁻¹, corresponding to 3000 mg.L⁻¹ of COD. Hajji et al. (81) studied the effects of bioagumentation stratigies in UASB reactors with methanogenic consortium for the removal of phenolic compounds.

2.4.3 Anoxic Wastewater treatment

Anoxic or denitrification technology has always been used for the treatment of nitrogen rich wastewaters. Methanol is generally used as the source of organic carbon in conventional denitrification processes. Recently anoxic denitrification processes are attracting wide attention for
the organic carbon removal due to some of the advantages of these processes over aerobic and anaerobic methods.

- High concentration of soluble electron acceptor (NO$_3^-$) can be maintained in the reactor.
- Denitrifying organisms are less sensitive to environmental inhibitory compounds as compared to anaerobic microbial consortia
- Formation of innocuous end products like nitrogen
- Maintaining conditions required for denitrification is easier as these organisms tolerate small amounts of oxygen
- Many phenolics are degraded under anoxic conditions.

Beccari et al. (82) studied the use of phenol as the carbon source in a denitrification reactor. In batch tests, they obtained maximum denitrification rates, which were only 40% of those reported with methanol.

Nutt et al. (83) applied fluidized bed fixed-film process to perform predenitrification-nitrification on a coke plant wastewater. Phenolic carbon represented 42-54% of raw wastewater filtered organic carbon (FOC) corresponding to a feed phenolic concentration of around 400 mgL$^{-1}$ and removal of 99.9 % of phenolics was observed.

Hu and Shieh, (84) reported investigations on the degradation of certain monocyclic aromatic compounds including phenol, induced by biofilms under anoxic conditions, with potassium nitrate as electron acceptor. They used upflow biofilter with glass beads as immobilization medium.
Godbole and Chakrabarty, (85) found an upflow anoxic fixed film-fixed bed (UAFFFB) reactor to be an effective system for the continuous long term biodegradation of phenol, resorcinol, catechol, cyclohexanol and cyclohexanone. The study highlighted the biochemical specificity acquired by a seed when exposed to different substrates for acclimation. For COD loading of 0.264 to 0.282 g COD/L-D, 42 to 71% phenol removal was found after 24hr.

Khoury et al. (86) studied anaerobic degradation of phenol in batch and continuous cultures by denitrifying consortium. $\mu_{\text{max}}$ obtained under anaerobic degradation of phenol under denitrifying conditions was 0.091h⁻¹.

Deshmukh et al. (87) employed resorcinol, a phenol derivative as electron donor in UAFFFB denitrification column. Performance evaluation studies indicated excellent resorcinol and nitrate removal under anoxic conditions. For COD loading of 6 g COD.L⁻¹.day⁻¹ and HRT of 8hr or more, it was found that COD and nitrate removal efficiency was more than 90%.

Fang and Zhou (88) conducted experiments in an upflow sludge blanket reactor treating wastewater containing phenol and m-cresol, 200 and 100 mg.L⁻¹ respectively and nitrate at various concentrations. Denitrifiers out competed methanogens for substrates for carbon source. They were able to use phenol and m-cresol as substrate without a carbohydrate co-substrate. Denitrifying 1g of NO₃⁻N required 3.34 g of COD.
Methanogenesis occurred only at COD/ NO$_3$-N ratios greater than 3.34. At the ratio of 5.23, over 98% phenol and 60% of $m$-cresol was degraded jointly by denitrifiers and methanogens with 1 day HRT.

This literature survey showed that an extensive work has been carried out on the application of aerobic and anaerobic wastewater treatment for phenolic wastewater treatment. However, only limited literature is available on the treatment of phenolic waste under anoxic conditions, especially using granular sludge.
References


