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Review article

BIODEGRADATION OF PHENOL-AEROBIC AND ANAEROBIC PATHWAYS

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ABSTRACT

For the maintenance of the quality of the environment it becomes necessary to degrade recalcitrant chemicals in a cost effective way either aerobically (Ortho/Meta pathways) or anaerobically (Benzoyl-coA/ Resorcinol/ phloroglucinol pathways). Ortho metabolism utilizes phenol hydroxylases enzyme to form cis,cis muconate or its derivatives as the end product, while in meta pathway the end product is 2-hydroxy muconic semi-aldehyde. The most well known key dihydroxyaromatic intermediates resulting from the biodegradation of aromatic compounds are catechol, protocatechuic acid and gentisic acid. These intermediates further undergo ring fission following the Krebs cycle to yield other metabolites, such as pyruvic acid, acetic acid, succinic acid and acetyl-CoA. Nitrate reducers differ in their strategies significantly from those used by sulphate-reducing or fermenting bacteria. Activities of various microbial phenol degraders are dependent on many environmental factors like pH, temperature and oxygen availability. Deviations in any of the required components lead to ceasing of microbial activities thereby decreasing the rate of phenol degradation. Anaerobic degradation requires at least four physiologically different microbial groups (trophic groups), hydrolyzing bacteria, fermenting bacteria, acetogenic bacteria and two types (*i.e*., acetoclastic and hydrogenotrophic) of methanogenic archaea. Anaerobic reaction always initiates with carboxylation and end products most commonly seen are Acetyl co-A, Succinyl Co-A. Methanogenic (anaerobic) bacteria are responsible for the conversion of phenol stoichiometrically to CH_4 and CO_2. Heterogeneous photocatalytic oxidation (HPO) process employing catalyst such as TiO_2, ZnO, *etc*. and UV light has demonstrated promising results for the degradation of persistent organic pollutants.

KEYWORDS: Phenol, aerobic degradation, anaerobic degradation, extradiol and intradiol fission, ortho/meta pathways, Phenol hydroxylases, Microbial fuel cell.

1. INTRODUCTION

After carbohydrates, aromatic compounds, found as lignin components, flavanoids, quinones, aromatic amino acids, or constituents of fossil fuels, are the most widely distributed class of organic compounds in nature. Thirty monoaromatics are on the EPA priority pollutant list and 11 of these compounds are among the top of hundred chemicals on the priority list of hazardous substances as published by the Agency for toxic substances and disease registry (Sridevi *et al*., 2012). The monoaromatic hydrocarbons benzene, toluene, ethylbenzene, and xylene, collectively called BTEX, are commonly found in gasoline and are highly volatile substances, due to their relatively high solubility and toxicity; they represent a significant health threat in contaminated environments. Biodegradation is the mineralization of the organic compounds to less harmful and hazardous products like CO_2, H_2O, NO_3 and other inorganic compounds which can be incorporated into natural biogeochemical cycles by the metabolic activities of the microorganisms. The nutritional requirements of microorganisms normally include nitrogen, phosphorus, potassium, sodium, calcium, magnesium, iron, trace elements and carbon (Ojumu *et al*., 2005). The massive mobilization of compounds in natural resources or the introduction of xenobiotics into the biosphere leads to unidirectional fluxes, which result in the persistence of a number of chemicals in the biosphere and thus constitute a source of contamination. Phenol, which possess hydroxyl residue at the first position of carbon within the aromatic ring, is a most commonly occurring aromatic organic compound, and is produced both naturally (in decaying dead organic matter, by natural decay of lignocellulosic material, in coal, forest and rangeland fires) and also synthetically. Free phenols are liberated into the environment as a result of metabolic intermediates during the degradation of various plant materials but emissions from the motor vehicles, as a major constituent of industrial effluents and also as a photo oxidant product of benzene, phenol forms a major environmental threat to mankind by increasing its concentration in addition to free phenols which is already present in the surroundings in nominal concentrations. Toxic and hazardous xenobiotics, which have a structure that is different from naturally occurring compounds, are more difficult to degrade and according to Alexander (1965) no natural organic compound is totally resistant to biodegradation provided that the environmental conditions are favourable, known as the principle of microbial infallibility. Phenols owing to their persistence in the nature are one of the most important groups of eco-toxins. Phenolic materials pose a formidable biochemical challenge due to the resonance energy that stabilises the

carbon–carbon bonds of aromatic rings. The European Union listed phenol among the 'substance undesirable in excessive amounts'. Bioprocesses owing to its innate and potent ability to mineralize organic compound pollutants in a less economical and environmentally friendly way, gains over most recently adopted chemical or physical techniques. It also possesses the capacity to destroy pollutants but only from one phase to another. As oxygen is readily available in the biosphere most of the microbes have developed certain in built pathways which can employ molecular oxygen for substrate activation in oxygenase reactions for aerobic biodegradation of aromatic compounds liberated in the environment through various processes whereas microbes also follow anaerobic pathways under methanogenic conditions (Chmielowski *et al*, 1965), or under nitrate reducing (Bakker,1977), sulphate reducing (Bak and Widdel, 1986) and iron reducing (Lovely and Lonergan, 1990) conditions.

Bioremediation involves processes like bioattenuation (ensures that contaminant concentration decreases with time at relevant sampling points) which, is a good clean-up technique for underground storage tanks contaminated with petroleum products, biostimulation (requires external manipulations if natural degradation is too slow) is effective in TCE contaminated groundwater and bioaugmenting by inoculation of bacteria with the desired catalytic capabilities to enhance bacterial degrading capacity. In addition, it has also been recently shown that complete degradation of monoaromatic hydrocarbons can also be coupled to the respiration of oxyanions of chlorine such as perchlorate or chlorate, or to the reduction of the quinone moieties of humic substances. Because of the lack of functional groups, hydrocarbons are largely apolar and exhibit low chemical reactivity at room temperature which makes them less susceptible for degradation. Relevance of this review lies in the fact that there are still certain areas which require great attention of microbial activity for biodegradation of many complex compounds like phenol, and research has to be expanded to the extent without any limitations. Areas like Olive oil production industries (a major food industry in the Mediterranean countries and now gaining widespread interest in countries like China and India) produces oil mill waste water which proves to be great contributors towards highly polluted effluents due to high phenolic contents ranging from 1.5 up to more than 8 g L^{-1} in addition to many other newer industries (Maza-Márquez, P., 2013) and no treatment using selected bacteriae have been reported. So, more mechanisms or pathways developed in microbes either in aerobic or in anaerobic conditions has to be studied for their effective utilization in these untouched areas of human activity.

1.1 Phenol

Phenol (Hydroxy-benzene or carbinol) 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 carbolic acid), though its composition was not known until 1841. Molecular weight of phenol is 94.11, the density is 1.072 and the boiling point is 181.9°C. It has a very strong odour (acrid odour) with an odour threshold of 0.04 ppm and a sharp burning taste. It is soluble in most organic solvents and its solubility in water is limited at room temperature, however above 68^0C it is entirely water-soluble. Phenol is obtained from coal tar as a disinfectant for medical (as mouthwash and sore throat lozenges, ATSDR, 1989) and industrial applications like petroleum refinery spent caustic which is alkaline, phenolic resins and plastics, leather and textile (nylon and other synthetic fibres) manufacturing, chemical and petrochemical plants, coke ovens, laundry operations, pulp and paper plants, rubber reclamation plants, Pharmaceutics and agro industrial operations, steel and varnish industries, pesticides (*eg*. phenoxyherbicides like 2,4 dichlorophenoxyacetic, dinoseb acid, or phenolic biocydes such as 4- chlorophenol or pentachlorophenol, (Laine *et al*,1996), tannery and smelting related operations and saline waste stream offer a great deal to the environment. Phenol evaporates more slowly than water and a moderate amount can form a solution. Potential renewable chemicals from bio-oil (derived from biomass pyrolysis) include phenolics and cyclic ketones for resins and solvents, levoglucosan and levoglucosenon for polymers and aromatic hydrocarbons for fuels and solvents (Maher, Bressler, 2007). Bio-oil was mainly composed of phenols, aliphatic hydrocarbons, aromatic hydrocarbons, furan derivatives, and some acid *etc*. Phenols in bio-oil include phenol, methyl phenol, ethyl phenols and methoxy phenols (Lei *et al*., 2011).Swine manure has also been detected to have Phenolic presence in a study by Leven *et al*, 2012. Mono- and oligonuclear aromatic compounds are important constituents of plant tissue, where they act as plant protectants that are either toxic to invading micro-organisms or polymerize upon exposure to air through radical-catalyzed polymerization reactions, to act as a wound seal. Aromatic amino acids add structural elements to the tertiary structure of proteins. In numerous coenzymes aromatic residues increase and define the reactivity of prosthetic groups in specific activation reactions and maintenance of reactive transition states (Schink Y, 2000). Phenol is a weak acid and in its ionized form, it is very sensitive to electrophilic substitution reactions and oxidations.

1.2 Positive impacts on human health

Eventhough, the ingestion of even minute levels of phenol (1g) is reported to be lethal for humans (Nuhoglu *et al.*, 2005) as they cause various health hazards, their (phenolic compounds) activities are not so when they are present naturally in the dietary sources such as the C6-C3-C6 in flavonoids-anthocyanins, dihydrochalcones, flavan-3-ols, flavanones, flavones, flavonols and isoflavones, tannins, phenolic acids, hydroxycinnamates and stilbenes and the transformation of plant phenols associated with food processing (for example, production of black tea, roasted coffee and matured wines). Dietary phenolics being the major dietary sources are currently of great interest due to their antioxidative and possible anticarcinogenic activities. Phenolic acids and flavanoids also function as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation. In addition, flavanoids and phenolic

acid components play important roles in the control of cancer and other human diseases. Antidiabetic activity of flavanoids and phenolic acids has been reported by several studies (Ghasemzadeh *et al.*, 2011). Oxidative stress is considered to be substantial, if not crucial, in the initiation and development of many current conditions and diseases, including: inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, arteriosclerosis and aging (Lukyanova *et al*., 2007). Oxidative stress plays a role in heart diseases,neurodegenerative diseases, cancer and in the aging process (Zima *et al*., 2001; Astley, 2003). Ghasemzadeh *et al.* (2011) reported that the isolated polyphenols from young ginger (*Zingiber officinale*) including quercetin, kaempferol, rutin gallic acid, were shown to inhibit the growth of human breast cancer cell lines (MCF–7 and MDA –MB –231). However, there is now emerging evidence that the metabolites of dietary phenolics, which appears in the circulatory system in n mol/L to low m mol/L concentrations, exert modulatory effects in cells through selective actions on different components of the intracellular signalling cascades vital for cellular functions such as growth, proliferation and apoptosis. In addition, the intracellular concentrations required to affect cell signalling pathways are considerably lower than those required to impact on antioxidant capacity.

1.3 Negative health impacts on human health

Exposure to phenol by any route can produce systemic poisoning with symptoms such as initial, transient CNS stimulation, followed rapidly by CNS depression. In recent years, a great attention has been paid to endocrine disrupting chemicals (EDCs) in environmental science research and more than 100 types of EDCs have been reported. Alkyl phenols (APs), as one important class of EDCs, have an evident estrogenic effect and have been widely detected in the environment (Wang *et al*, 2007). Phenol is corrosive and causes chemical burns at the contact site. When exposed to skin it can cause necrosis. Coma and seizures can occur within minutes or may be delayed up to 18 hours after exposure. Certain other symptoms are nausea, vomiting, diarrhoea, methanoglobinemia, haemolytic anaemia, profuse sweating, hypotension, arrthymia, pulmonary oedema, and tachycardia. Phenol is absorbed mainly from the lungs, however owing to its low volatilization its health hazards are limited. Phenol vapour and liquid penetrate the skin with absorption efficiency approximately equal to the absorption efficiency by inhalation. Both in vivo and in vitro tests have shown covalent binding of phenol to tissues and plasma proteins and some phenol metabolites also bind to the proteins. Urinary (renal) excretion is the major route of phenol elimination in humans and animals. A minor part of phenol is eliminated n expired air and faeces. The natural presence of phenolic compounds in food and drug metabolites makes biological monitoring impossible. Due to these reasons and other adverse health and environmental impacts it becomes a high essentiality to develop newer technologies which is less economical and environmental friendly to a great extent so that the harmful by products that are produced as a result of varied metabolic pathways induced within many microbial strains which can degrade these highly complex aromatic organic compounds could be bought within the permissible limits in the environment thereby leading to the betterment of the mankind.

MICROBES IN DIVERSE ENVIRONMENTAL CONDITIONS

Extremophilic microorganisms are adapted to grow and thrive under these adverse conditions. Hydrocarbon degrading extremophiles are thus ideal candidates for the biological treatment of polluted extreme habitats. Cold adapted psychrophilic and psychrotrophic microorganisms being widespread in earth's biosphere, where temperature is below 5^0C (as they are able to grow in 0^0C, which implies that, their ambient temperature often coincides with their growth temperature range, Margesin and Schinner 1999a). Psychrophiles have an optimum growth temperature of ≤15°C and do not grow above 20°C, whereas psychrotrophs (cold-tolerant) have optimum and maximum growth temperatures above 15°C and 20°C, respectively (Morita, 1975). Significant numbers of cold-adapted hydrocarbon degraders were even found in contaminated soils from the most northerly inhabited station in the world, Canadian Forst Station-Alert (Whyte *et al*., 1999); the temperature threshold for significant oil biodegradation is around 0°C (Siron *et al*., 1995), so the winter period was found to be critical for an oil spill in Arctic/sub-Arctic environments because of the reduced biodegradation under icy conditions. Psychrophilic strains with high oil-oxidizing and bioemulsifying activities were also described by Chugunov *et al*. (2000). Intrinsic hydrocarbon bioremediation was also demonstrated in a shallow aquifer underlying a natural gas production (Gieg *et al*., 1999). Changes were monitored over 4 years. All BTEX compounds were biodegraded under sulphate-reducing conditions; toluene was also degraded under methanogenic conditions. Other authors (Weiner and Lovley, 1998) noted the persistence of benzene in the sulphate reduction zones of petroleum-contaminated aquifers. This benzene persistence was attributed to the lack of benzene-oxidizing sulphate reducers in the aquifer sediments, rather than to the inability of such microorganisms to grow and metabolize under freshwater conditions. Haloalkaliphiles are bacteria that thrive in saline and alkaline environments such as soda lakes. The haloalkaliphilic bacteria *H. campisalis* degrades phenol and catechol in alkaline (pH value 8-11) and saline environments (0-150g/l NaCl) (Taghreed *et al.*, 2012) or atleast 1M NaCl (approx. 6% w/v) for growth and grow optimally at NaCl concentration above 3M (Kushner 1978, Grant *et al.*, 1998), whereas microorganisms that are able to grow in the absence of salt are known as halotolerant. Halophilic archaea maintain an osmotic balance with the hypersaline environment by accumulating high salt concentrations, which requires salt adaptation of the intracellular enzymes. Eubacteria are more promising degraders than archaea as they have a much greater metabolic diversity. Their intracellular salt concentration is low, and their enzymes involved in biodegradation may be conventional (*i.e.* not salt-requiring) enzymes similar to those of non-halophiles (Oren *et al*., 1992). There is an inverse relationship between salinity and solubility of PAHs (Whitehouse 1984). A comparison of the effect of

temperature on PCB removal by mesophilic and cold-adapted degraders demonstrated that the PCB-degrading enzyme system of the Arctic bacteria is cold-adapted (Master and Mohn 1998).Various studies indicate that bioremediation process has to still reach certain heights as there are still problems in tackling many pollution affected sites like oil polluted desert soil areas because being a vast ecosystem it is practically impossible to inoculate the affected site with efficient degraders. Here, soil moisture is a necessary criterion for the development of these degrading microorganisms and irrigating the site with seawater adds further to the problem. The reason being, high salinity can cause soil moisture to evaporate and cause the deposition of the salt whereas irrigating the area with large volume of freshwater can cause leaching of salts which then leads to the reduction in soil salinity (Nair *et al.*, 2008). Radwan *et al*. (2000) proposed vegetation as a feasible approach for bioremediation of oil-polluted desert soil. Dispersants are often used to emulsify the oil slick in order to improve bioavailability and to enhance oil biodegradation. The rhizosphere of the investigated wild desert plants and crop plants (*Vicia faba, Lupinus albus*) contained more hydrocarbon-utilizing bacteria than the soil alone. This effect was much more pronounced for plants growing in oil-polluted than in uncontaminated soil. Taylor *et al*., 1998; recommends the coupling of bioremediation to in situ underground stripping (DUS), a primary decontamination effort for the rapid treatment of contaminated soil at significant depths. Although volatile organic compounds such as BTEX aren't completely removed by vaporization, the temperature elevation (50-70^{0}C for 60 days) caused as a result can enable thermophilic bacteria to metabolize remaining low levels of residual BTEX. The bioremediation process is terminated by lowering the temperature below 40°C. Such an in situ follow-up treatment could also be applied to fuel-contaminated plumes subjected to thermally enhanced vapour stripping as a primary treatment method, or as a stand-alone method, when the initial concentration of VOCs is low and the subsurface volume to be heated is small.

Acidophiles are metabolically active in highly acidic environments, and often have a high heavy metal resistance (Norris and Johnson 1998). Remarkably, aliphatic organic acids, which are thought to be toxic to acidophiles (Alexander *et al*., 1987), were utilized as substrates for energy and growth. Stapleton *et al*. (1998) reported the biodegradation of aromatic hydrocarbons and PAHs in extremely acidic environments. Genes that encode enzymes involved in biodegradation of aromatic hydrocarbons can be acquired and expressed in some heterotrophic acidophilic bacteria (Quentmeier and Friedrich, 1994). Alkaliphiles are defined as organisms that have their optimum growth rate at least 2 pH units above neutrality. Alkalitolerants are able to grow or survive at pH values above 9, but their optimum growth rate is around neutrality or less (Kroll, 1990). In a study, 14 isolates, are able to utilize phenol (500 mg l^{-1}) at pH 10, were obtained from sediments of a highly saline and alkaline (average pH 10.5) lake in Lonar (India) (Kanekar *et al*. 1999). Four of these alkaliphilic bacteria (*Arthrobacter sp., Bacillus cereus, Citrobacter freundii, Micrococcus agilis and Pseudomonas putida biovar B*) were used for bioremediation of waste effluents arising from industries that manufacture methyl violet and cumene-phenol, using phenol as a major raw material.

Barophiles (piezophiles) are microorganisms that require high pressure for growth, or grow better at pressures higher than atmospheric pressure (Prieur and Marteinsson 1998). Pollutants with densities greater than that of marine waters may sink to the deep benthic zone, where the hydrostatic pressure is notably high. Eventhough, studies show low microbial activity certain strains have been isolated *viz*., *Pseudomonas, Aeromonas* and *Vibrio* spp. (Alexander, 1999)

PHENOL IN THE ENVIRONMENT

Phenol is a major pollutant included in the list of EPA (1979). As per central pollution control board set the minimum permissible level for phenol in environment as 0.05 to 0.1mg/l (Das *et al*., 2012). Phenol crystals are hygroscopic and turn pink when exposed to air. The range of phenolic concentration in waste water may vary from 10 to 300 mg/l but this can rise up to 4.5 mg/l in highly polluted waste waters. Phenol and its derivatives are either toxic or lethal to fish at concentration of 5-25 mg/l, which imparts an unpleasant odour to drinking water even at much lower concentration of 2µg/l. WHO has set a guideline of 1µg/l to regulate phenol concentration in drinking waters. US Environmental Protection Agency has set a water purification standard of phenol concentration less than 1µg/l in surface waters while phenol and phenolic compounds are found at concentration of less than 1mg/l in nature but in industrial effluents are known to produce phenol in the range of 10mg/l to well over 10,000 mg/l (106 µM) (Fedorak and Hrudey, 1988), whereas domestic wastewater contains between 0.1mg/l 1mg/l (1 to 10µM) Hunter, 1971. The European Council Directive has set a limit of 0.5 µg/l to regulate the phenol concentration in drinking waters (Taghreed, 2012). European union is now following commendable work by emphasizing on complete elimination of hazardous compounds in surface waters by 2015. Phenol concentration in the ground water surrounding sites where raw creosote is used as wood preservatives especially for railroad ties, telephone poles and piers is reported to as high as 30mg/l (320 µM, Paula,2000)

IMMOBILIZATION

It has been found that bacteria's suffer from substrate inhibition, whereby growth and consequently phenol degradation is inhibited at high phenol concentration. [Hill *et al* (1975), Loh *et al* (2000)]. Roya *et al*., 2011 detected that the intense substrate inhibition adversely affected the biodegradation process at the phenol concentration of 1000 mg/l; the residual phenol in the medium reached to 901 mg/l within the entire time of experiment. Various methods have been proposed to overcome substrate inhibition in order to treat high concentration phenolic waters which includes adapting the cells to higher phenolic concentration, immobilization of the cells, use of more genetically engineered microorganisms or else supplement with addition carbon sources like Glucose (Das *et al.*, 2012) or yeast extract so as to attain maximum cell growth. It has been noted that addition of yeast extract

raised the affinity of *Pseudomonas putida* for phenol (Armenante, 1995), where phenol degradation is followed using meta-pathway). In a study conducted by Roya *et al.* (2011) it was found that adaptation of immobilized cells led to slightly shorter time for complete phenol removal in the range of 100-700 mg/l. At the phenol concentrations of 1000 mg/l, immobilized culture could improve phenol removal efficiency to about 40%.

According to (Loh *et al*, 1998) addition of conventional carbon source glucose substantially enhances the cell density. Various other studies like Topp *et al.* (1988) also demonstrate that addition of non-toxic compounds may stimulate the viability of the cells and increase the degradation. It was proposed that the presence of a more metabolisable carbon source promotes more rapid growth and multiplication. Free bacterial cells for waste water treatment in activated sludge processes creates problems such as solid waste disposal while immobilized microorganisms(trapping them on solid support particles such as alginate polyacrylamide, Chitosan-a natural non toxic biopolymer, diatomaceous earth, activated carbon, sintered glass, polyvinyl alcohol and polymeric membrane are capable of effective treatment and maximum degradation capacity with little sludge formation due to high surface area available for bio film formation resulting in high biomass concentration of 30-40 g/l compared with 1.5-2.5g/l for activated sludge systems.(Taghreed, 2012.). Immobilized method being non-toxic to the cells, inert and being more stable to shock loadings, can efficiently tolerate high concentrations of phenol and wider changes in temperature and pH, and is the more practical method for phenol degradation. Thus a good support material for biomass immobilization should be rigid, chemically inert, and inexpensive. It should bind the cells firmly and should have high loading capacity and looses structure for less diffusion limitations (Taghreed, 2012).

PHENOL DEGRADATION

The observed toxicities by Fang and Chan, (1997) were in the following descending order: cresols > phenol > hydroxyphenols/phthalate > benzoate. Toxicity thus increased with increasing hydrophobicity of the functional group. According to L.Leven *et al.*, 2005, the observed order of degradation efficiency among cresol is as follows: p-cresol > m-cresol >> o-cresol. The degradation of p-cresol is initiated by oxidation of the methyl-group, which is the probable reason for its rapid degradation. United States environment protection agency has classified p-cresol as pollutant of group C (possible human carcinogens) and listed it as priority pollutant. Susceptibility of phenolic compounds to biodegradation is given as: para-nitrophenol > 2, 4- dichlorophenoxyacetic acid > pentachlorophenol (Ingerslev, Nyhlom, 2000).

Howard (1989) reported that phenol degradation rates suggest rapid aerobic degradation in sewage (typically 905 with an 8 h retention time), soil (typically complete biodegradation in 2-5 days), fresh water (typically biodegradation in <1 day), and sea water (typically 50% in 9 days), however the rate is comparatively slow when anaerobic biodegradation is considered. Toxicity prevents or slows metabolic reactions. Microbial cells cease to function when at least one of the essential steps in their numerous physiological processes is blocked. The blockage may result from gross physical disruption of the cell structure or competitive binding of a single enzyme essential for metabolising the toxicant (National Research Council, 1993). Soil types affect the rate of mass transport of nutrients, pollutants, and air, water and pH adjusters. This effect on mass transport in return affects the degradation process (Talley and Sleeper, 1997). Strain ability to cope with the toxic effects of phenols was reflected on their growth rates and was proportional to the applied phenol concentration which was well demonstrated in the work carried out by Maza-Márquez *et al*. (2013). In their study it was shown that when the phenol concentration was zero, the strains grew rather well (may be due to the absence of substrate inhibitory effect) but as the phenol concentrations gradually increased toxic effects were recorded for the majority of strains, while only few other maintained almost same growth rates as recorded in zero phenol concentration. Beyond a certain inhibitory initial concentrations, the biomass growth yield and specific growth rate tend to decrease because of increased substrate toxicity at higher initial substrate concentrations. This decline trend shows that phenol, p-cresol, and resorcinol are inhibitory substrates (S. Kumar *et al*., 2013). Competitive inhibition type of substrate interaction was found between phenol and p-cresol, phenol and resorcinol.

Phenol degradation mainly implies mineralization or breakdown of various organic compounds. So, recently biodegradation through photocatalytic activity with semiconductor particles acting as photocatalysts have gained the focus. In the photocatalytic oxidation process, organic pollutants are destroyed in the presence of semiconductor photocatalysts (*e.g*., TiO_2, ZnO) an energetic light source, and an oxidizing agent such as oxygen or air (Ahmed *et al*., 2011). Particle size is of primary importance in heterogeneous catalysis, because it is directly related to the efficiency of a catalyst through the definition of its specific surface area. The heterogeneous photocatalytic process is initiated when a photon with energy equal or greater than the band gap energy (Ebg) of the photocatalyst reaches its surface, resulting in the generation of mobile electrons in the higher energy conduction band (Ecb) and positive holes in the lower energy valence band (Evb) of the catalyst. The photocatalytic reaction proceeds via a series of chemical events, through the utilization of both the electron–hole h^+ for oxidation processes and eventually to the capture of the e^- electron for reduction processes, as well as potential formation of super oxide anions and hydrogen peroxide from oxygen. These facts allow mineralization of organic species (H. Benhebal *et al*., 2013). Photocatalytic activity of catalyst was evaluated by measuring the photodegradation of phenol and benzoic acid. It has been understood from various studies that parameters such as pollutant concentrations, viable biomass, concentrations, existence of inhibitor, temperature, pH, microbial completion and adaptation are the most important factors that affect the phenol degradation and its rate depends on the period in which the culture was adapted to phenol. As a general rule, most of the halo aromatics are degraded through the formation of the respective halocatechols, the

ring fission of which takes place via ortho-mode. On the other hand, most of the non halogenated aromatic compounds are degraded through meta pathway (Nair *et al.*, 2008). Gladyshev *et al.* (1998) reported that biodegradation of phenolic compounds is known to increase at higher concentrations of inorganic nutrients, while it is inversely affected by higher concentrations of organic nutrients. The process of Co-metabolism is an important example of the influence of substrate interaction during the biodegradation of pollutants. Co-metabolism is defined as the degradation of a compound only in the presence of another organic material that serves as the primary growth substrate. (Gladyshev, 1998 and Annadurai, 2008). For the past 50 years Chlorinated aliphatic .solvents, in particular the chlorinated ethenes tetrachloroethene (PCE) and trichloroethene (TCE), have been widely used especially in dry cleaning operations and as metal degreasers. Improper handling, storage, and disposal have turned these relatively water-soluble compounds into major groundwater contaminants. Under aerobic conditions, TCE (tetrachloroethene), DCE (1, l-dichloroethene), and VC (Vinyl chloride) can be transformed by the strategy cometabolism. This was first demonstrated with methanotrophic bacteria, whose soluble methane monooxygenase enzyme was found to gratuitously convert TCE to organic acids that can then be readily mineralized by other, heterotrophic bacteria (Patel et aI., 1982). Subsequently, aerobic bacteria capable of degrading aromatic compounds, particularly phenol and toluene-degraders, were shown to achieve cometabolic TCE degradation as well. Phenol has been claimed as a good growth substrate in the biodegradation of chlorophenolic compounds because of its similar chemical structure and lower toxicity. Although glucose has been a widely used conventional carbon source in biotransformation studies, it has never been used in the cometabolic transformation of 4-CP by Comamonas Testosteroni (M. Tobajas *et al*., 2012) but it can support cometabolism of 4-CP through the generation of NADH. A large numbers of natural and synthetic organic compounds are degraded 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 part of the organic carbon is synthesised into cellular material. Such conversions can take place either in aerobic environments, where oxygen acts as the terminal electron acceptor or also in anaerobic environments where nitrate, sulphate, carbon dioxide, other inorganic elements, or the organic compounds themselves serve as the terminal electron acceptor. Study of Abdulla *et al.* (2010) on Pseudomonas putida CP1 and A (a) indicates that increase of cell growth with the increase of incubation time is proportional to the substrate utilization. In general, a fumarate addition reaction is used as the initial activation step of the catabolic process of the corresponding monoaromatic hydrocarbon compounds. In the case of toluene, fumarate addition to the methyl group mediated by benzylsuccinate synthase appears to be the universal mechanism of activation and is now known to be utilized by anoxygenic phototrophs, nitrate-reducing, Fe (III)-reducing, sulfate-reducing, and methanogenic cultures. Many of these biochemical pathways produce unique extracellular intermediates that can be utilized as biomarkers for the monitoring of hydrocarbon degradation in anaerobic natural environments (Chakraborty *et al.*, 2004).

Kerala in India, popularly known as ‘God’s Own country” is known worldwide for its beautiful backwaters and estuaries. Retting is a native technology prevalent along the coast of Kerala as the indigenous practice for the processing of coconut husks. Retting process releases various aromatic compounds into the estuaries and phenol is one among them, which is known to have pronounced effects even at very low concentrations. Although chemical methods are in trend for removal of phenol, biodegradation methods have proven to be more efficient. Retting of coconut husk is basically a biological process involving the combined action of micro flora present in the retting environment. The retting process is anaerobic and thus requires the involvement of anaerobic microorganisms to separate fibres from husk. Such anaerobic consortium can be used in formulating bioremediation of phenol both for coir industries and also for other industrial effluents containing phenol. In the process of retting, the steeped husks imbibe water and sinks downward in the water. Normally it takes two to three months for microorganisms to act upon the husk. This lag phase or delay is due to phenolic substances present in the husk (Aziz, 1978). Polyphenols from the husks are constantly leached out into the surrounding steep liquors during the course of retting and the relatively high percentage of such polyphenols in coconut husks are the very reason for the delay in the completion of the retting process. These substances check the proliferation of the microorganisms and retard their metabolic activity and henceforth the bioremediation of polyphenols in the retting areas. In contact with soil, the mobility of phenol and phenolic substances becomes restricted due to the adsorption to solid surface, where they interfere with ecosystem equilibrium through selective toxicity affecting biogeochemical pathways of organic matter and nutrient recycling (Bronner and Goss, 2011b). The biomass growth yield and the substrate degradation are not directly proportional to each other. Substrate degradation takes place even though the biomass growth yield is low, because the consumed substrate is utilized for more energy generation to be utilized for higher maintenance of microbial cells at enhanced concentration of the toxic substrates like phenol, resorcinol, and *p*-cresol, causing the inhibition to biomass growth and to their own degradation. Hence, the concept of energy expenditure for maintenance of cells is needed to provide proper description of biodegradation dynamics (S. Kumar *et al*, 2013). Various literatures have shown that physic-chemical characteristics play a great role in deciding the growth or population of many microorganisms.

1. EFFECT OF pH

At low(4.0) or high (9.0) pH values acids or bases can penetrate into cells more rapidly, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells (Robertson, 1992). The follow-up of the medium pH can

be an indicator of the phenol degradation and one of the factors significant in the success of the biological treatment. A slight reduction is observed as biomass grows and pH variation increases when the initial phenol concentration increases. The decrease in pH suggests that biological degradation of phenol occurs and with a stable pH of about 7 (and a sufficient oxygen supply) phenol was successfully degraded. The pH significantly affects the biochemical reactions required for phenol degradation. For Aksu and Gonen, (2006) pH affects the surface charge of the cells of the activated sludge biomass. The surface charge of biomass is predominantly negative over the pH range of 3–10. Phenol could be expected to become negatively charged in phenoxide ion above a pH of 9. Below a pH of 3, the overall surface charge on cells becomes positive due to isoelectric point of activated sludge so the electrostatic attraction between phenol and activated sludge biomass will be insignificant. (B. Marrot, 2006).

2. EFFECT OF TEMPERATURE

Growth rate in general roughly double for each 10^0c rise in temperature within the usual mesophilic range of 10-30^0c, although it shows no change between 35-40^0c, and denaturation of proteins at higher temperature slows growth rate for mesophiles. According to Annadurai *et al.*, 1999 and Chithra (1995) phenol degradation is a temperature dependent process as demonstrated in their study that when the temperature increased from 30-34^0c no phenol degradation was observed due to cell decay. Various studies confirms that for maximum microbial activity work should be operated at the lower mesophilic range with an optimum temperature of around 35^0c or in the thermophilic range with a temperature optimum of 55-60^0c. A variation of 5^0c may cause a decrease in phenol degradation rate of at least 50% at the lower end and almost 100% at the higher end. The difference between phenol removal efficiency at 30^0c is probably due to the higher production of metabolites at this temperature. Moreover, at this temperature, the degradation rate seems better for free than immobilized cell system (1.45 times higher) (B. Marrot, 2006). Bioavailability and solubility of less soluble hydrophobic substances, such as aliphatic and polyaromatic hydrocarbons, are temperature dependent. A temperature increase (decrease) affects a decrease (increase) in viscosity, thereby affecting the degree of distribution, and an increase (decrease) in diffusion rates of organic compounds. The increased (decreased) volatilization and solubility of some hydrocarbons at elevated (low) temperature affects toxicity and allows biotransformation with high (low) substrate concentrations (Müller *et al*., 1998; Whyte *et al*.,1998; Niehaus *et al*., 1999). Optimal growth of extreme thermophiles and hyperthermophiles occurs at 70–80°C and above 80°C, respectively (Stetter, 1998). Thermophiles, predominantly bacilli, possess a substantial potential for the conversion of environmental pollutants, including all major classes (Müller *et al*., 1998). Various degradation studies have shown that no or very slow degradation, often with a long lag phase, occurs for both phenol and p-cresol at the thermophilic temperature, while both compounds are quickly converted to methane at the lower temperature. Thus, lowering of temperature from a thermophilic to a mesophilic temperature triggers the degradation of both phenols and p-cresol due to enhancement of the degradation rate by the microbial consortia. Temperature not only influences the community structure in general, but also the methanogenic consortia degrading phenol (Levén *et al*., 2012). According to Li *et al*, 2010 the maximum growth rate was obtained at 50 mg/L phenol; below this concentration, growth appeared to be suboptimal due to substrate limitation and above this concentration; growth was increasingly inhibited due to substrate inhibition.

3. Microorganisms related to phenolic degradation

Microorganisms known to degrade phenols and related organic compounds (Kumaran, 1993)

Compounds	Microorganisms
Hydrocarbons	*Pseudomonas putida, P.aeruginosa, P. stutzeri, Flavobacterium, Achromobacter, Bacillus, Vibrio, Spirillum, Candida, Nocardia*
Naphthalene, Salicylate, Pthalates, Paraffins	*Beijerinkia, P.aeruginosa, P.putida, Micrococcus, Nocardia, Antrhobacter paraffineus*
Phenolic compounds	*Alcaligenes, Acinetobacter, Achromobacter, Azotobacter, Bacillus subtilis, B. cerus, Brevibacterium, Flavobacterium, P. aeruginosa, P. cepacia, P. fluorescens, P. stuzeri, P. putida, Candida tropicalis, Trichosporon cutaneum, Nocardia*
Lignin and its derivatives	*Aspergillus niger, Phanerochaete chrysosporium, Polycysticus versicolor, Pleurotus ostreatus, Coriolus versicolor, Chaetomeium piluliferum, Nocardia sp., Fusarium, P. fluorescens, P. putida, P. testersteroni, P. acidovorans, B. subtilis, Acinetobacteri, Candida albicans, Alcaligenes, Trametes sp., Penicillium sp.*

The strain degrading phenol and other aromatic derivatives to a greater extent within a relatively short time are designated as efficient degraders among the isolates. . Some phenolics have been reported to decrease chemical oxygen demand (COD) and nitrogen and phosphorous uptake in various microorganisms (Kargi *et al*., 2005), suggesting that phenolic pollution may also have effects on microbial communities. Gram-positive species perform better in terms of being able to utilize higher initial concentrations of phenol in pure liquid culture without growth inhibition. This trend holds true for the phenol degradation in soil, as well (Djokic *et al*., 2013). Better tolerance and degradation of the higher initial concentrations of phenol may be due to the differences in the constitution of cell structures with sturdier cell wall, thereby allowing improved mechanism to cope and adapt to the stress of the presence of higher concentrations of hydrophobic compounds in addition to their spore forming capacity which helps them to persist in many harsh conditions. The mesophilic species are most often present in the degradation of phenol and chlorophenol viz.,*Pseudomonas, Flavobacterium, Achromobacter,*

Rhomobacterium, Azobacter, Micrococcus, Bacillus alkaligenes, Arthrobacter,Ycobacterium, Aeromonas, Nocardia and Lophomonas (Jianhua Wang *et al*., 2012). Strain Bacillus sp. PS11 was previously isolated and characterized as a strain which utilizes high amount of phenol (up to 1400 mg l−1) in liquid culture without apparent inhibition of growth (Djokic *et al.*, 2011). Two groups of degrading bacteria that are mainly specified and used are *Rhodococcus spp*. and *Pseudomonads* like *Pseudomonas putida.* P. putida seem to have the highest degradative potential. That is why a great number of studies upon the degradation of phenols by these bacteria have been done. Leonard and Lindley (1998) have described the biodegradation or metabolism of phenol by *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas picketti* and *Alcaligened eutrophus* respectively via the meta cleavage pathway, while Paller *et al*. (1995) described the biodegradation of phenol by *Trichosporon cutaneum*, *Rhodotorula rubura* and *Acinetobacter calcoacetium* respectively via the ortho cleavage pathway. In *Pseudomonads*, many of its induced enzymes are non-specific and its metabolic pathway contains a high degree of convergence. The convergence of catabolic pathways allow for the efficient utilisation of a wide range of growth substrates while the non specificity of the induced enzymes allows for the simultaneous utilisation of several similar substrates without an excess of redundant genetic coding for enzyme induction (Hutchinson and Robinson, 1988). However, Ryan *et al.* (2007), in contradiction to many other validated results or observations, reported in their review study on *R. pickettii* that though *P. putida* and *P. fluorescens* are of the best characterized for phenol biodegradation, they have drawbacks that may limit their use *P. fluorescens* has been demonstrated to cause fin rot in fish and *P. putida* has also been shown to cause disease in fish. The use of these bacteria as biodegraders is, therefore, not advisable as environmental release could lead to environmental damage, such as disease in plants and depletion of fish stocks, and the potential cause of disease in humans. The use of these organisms could also raise public concern. However Results obtained by Das *et al.*, 2012 suggests that under optimum conditions of temperature i.e. 30°C, pH 7.0 and 150 rpm *Pseudomonas aeruginosa* proved to be an efficient host for phenol degradation with a degrading potential of 1250ppm.

Dechloromonas strain RCB has been shown to be capable of anaerobic degradation of benzene coupled to nitrate reduction. With nitrate as the electron acceptor, strain RCB degraded benzene and toluene concurrently when the hydrocarbons were added as a mixture and almost 92 µM total hydrocarbons were oxidized within 15 days. In addition to nitrate, strain RCB could alternatively degrade benzene both aerobically and anaerobically with perchlorate or chlorate [(per) chlorate] as a suitable electron acceptor. Furthermore, with nitrate as the electron acceptor, strain RCB could also utilize toluene, ethylbenzene, and all three isomers of xylene (ortho-, meta-, and para) as electron donors (Chakraborty, 2005). It has been demonstrated that extracellular enzymes produced by Enterobacterium and Clostridium species permit complex organic matter degradation (Wust *et al*., 2011). Enterobacteriaceae, Bacillaceae and Micrococcaceae, are universally found in soils of temperate regions; among them, various species had been described for their ability to degrade aromatics such as PHA, dioxins and phenolic compounds (Zhao *et al*., 2012). Desulfotomaculum has also proven to be phenol degraders as its degradation pattern is similar to that of C. phenolicus, which can only convert phenol into benzoate in the presence of unknown growth factor (Qiu *et al*., 2008). Furthermore, only three phenol-degrading bacteria have been isolated from methanogenic environments; Sedimentibacter hydroxybenzoicum (previously known as Clostridium hydroxybenzoicum), Cryptanaerobacter phenolicus and Syntrophorhabdus aromaticivoran (Levén *et al.*, 2010). Reviews show that much lesser microbes have been isolated from seawater and among a few isolated ones Acinetobacter sp. EBR01 proves to be the most efficient category. From this they concluded that a kind of Acinetobacter strain that has organic-compound-degrading activities in terrestrial and freshwater environments seems to accumulate in marine sediments and is ingested by the benthos community. More innovations should be achieved in developing mathematical models to explore the activity of Acinetobacter sp. EBR01 in future (Kobayashi *et al*., 2012). The *Acinetobacter, Alcaligenes, Inquilinus* and *Methylobacterium* isolates reported are able to degrade 4-t-octylphenol in a study by Tuan *et al*.,2011.

4. Aerobic process

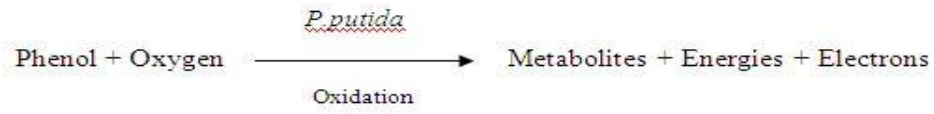
Molecular oxygen is a key factor and an important cofactor required for the microbial degradation of many compounds especially aromatic hydrocarbons, phenolic and other ring compounds. In aerobic bacteria growing on hydrocarbons, O_2 is not only the terminal electron acceptor for respiratory energy conservation, but also an indispensable reactant in the activation mechanism. (Aerobic biodegradation has been studied since the beginning of the 1900s. Generally bacterial respiration doesn't appear to be affected above a critical dissolved oxygen (which is the concentration at which the respiration rates of the cells is one half of the maximum rate observed at the saturating levels; it is generally low for the dispersed cultures than flocculent cultures and is typically in the range of 0.5mg/l, Gaudy *et al.*, 1980).

Key enzymatic reactions of aerobic biodegradation are oxidations, catalyzed by oxygenases. Oxygenases are oxidoreductases that use O_2 to incorporate oxygen into the substrate. Aerobic biodegradative organisms need oxygen at two metabolic sites, one at the initial attack of the substrate and another at the end of the respiratory chain. The key enzymes of the biodegradation of aromatic substrates are induced and synthesized in appreciable amounts by microorganisms only when the substrate or structurally related compounds are present in growth media (N. Pradhan *et al*., 2007). When glucose added is being completely utilised, microbes starts to nurture on phenol as its carbon source whereas, O'Sullivan (1998) reported an inhibitory effect on the removal of phenol and mono-chlorophenols by a mixed microbial population in the presence of glucose. This study reported a suppression of phenol removal in the presence of glucose. The presence of glucose exerted repressive effects on phenol

removal by *P. pictorum*. Reduction of phenol removal rates in the presence of various concentrations of glucose was also reported for heterologous populations (A. F. Rozich, 1986). A glucose concentration of 0.5% repressed the induction of phenol oxidation though glucose did not fully repress utilization of phenol (Chakraborty *et al*., 2010). The degradation rate of phenol in the presence of glucose was lower than in simple phenol solution, indicating that phenol degradation by *P. chrysogenum* was delayed in the cultures with 3% glucose unlike results obtained by Santos *et al*. (2003) and Khaled (2006) in their respective studies. A cross inhibitory pattern was also described for phenol and glucose by *Pseudomonas putida* ATCC17514 (Wang *et al*., 1996). The authors observed that the reduction of specific substrate utilization rates indicated that the two substrates were involved in a cross inhibitory pattern.

During acclimatization process certain enzymes in the bacteria are induced so that they are available for taking part in the metabolism reaction. This is much more important when dealing with toxic compounds such as phenol at high concentrations. Phenol hydroxylases enzyme represents an important group of enzymes, which shows organizational similarity and low sequence identity with the multicomponent mono- and dioxygenases involved in the degradation of toluene, benzene, naphthalene, and methane. Five proteins appeared to be necessary for the production of this multicomponent enzyme. One of these, P5, is a flavin adenine dinucleotide (FAD) containing electron transfer component with an iron sulfur center (Paula, 2000). Phenol hydroxylases ranging from simple flavoprotein monooxygenases to multicomponent hydroxylases, as well as the genes coding for these enzymes, have been described for a number of aerobic phenol-degrading microorganisms. The mono oxygenase phenol hydroxylase of the *Trichosporon cutaneum*, *Pseudomonas pickett*, *Bacillus stearo thermo phylus* BR219 and some species of acinetobacter and alcaligenes are monocomponent flavoproteins (an oligomeric protein) (Kim *et al.,* 2002; Neujahr and Gaal, 1973), while the mono oxygenase phenol hydroxylase of *Pseudomonas CF600* and *Acinetobacter radioresistens* (Shingler *et al*., 1989) are multicomponent proteins (monomeric iron transfer flavoprotein). Multicomponent aromatic mono oxygenases contain at least two components.

During the first step of the aerobic pathway for the biodegradation of phenol, molecular oxygen is used by the enzyme phenol hydroxylase (a NADPH dependent flavoprotein) to add a second hydroxyl group in ortho-position to the one already present. Phenol hydroxylases have been described in extracts of *T.cutaenum* (Neujhar and Gaal, 1973), *C. tropicalis* (Neujhar *et al*., 1974) and . The reaction requires a reduced pyridine nucleotide ($NADH_2$). The resulting catechol (l, 2 dihydroxybenzene) molecule can then be degraded via two alternative pathways, depending on the responsible organism. In the ortho- or β-ketoadipate pathway [the first evidence for the production of β-ketoadipate was reported (Evans, 1947; Kilby, 1948), was isolated from coal gasification wastewater (Happold and Key, 1932). The organism was later identified as Acinetobacter calcoaceticus.], the aromatic ring is cleaved between the catechol hydroxyls by a catechol 1, 2-dioxygenase (intradiol fission). Then the resulting cis,cis muconate or its derivatives (depending on whether the catechol is substituted or not) is further metabolized, via β-ketoadipate, to Krebs cycle intermediates (Basha *et al*,2010) or in TCA cycle resulting in CO2, metabolites and energy (Das *et al*,2012). In the meta pathway, ring fission occurs adjacent to the two hydroxyl groups of catechol (extradiol fission) and the end products are 2-hydroxy muconic semi-aldehyde which also enters TCA cycle. Ortho pathway is the most productive pathway for the organism as it involves less expenditure of energy.



Great varieties of aromatic substances are transformed by mono- and dioxygenases to a few key intermediates by the introduction of hydroxy groups and the removal of certain substituents (channeling reactions). The most prominent intermediates are catechol, protocatechuate, and gentisate, which are subject to ring cleavage in a further oxygenase-dependent step, either between or vicinal to the hydroxyl groups of the aromatic ring (Dagley 1971; Schlegel 1992). Thus an unsaturated, open-chain carboxylic acid is formed which undergoes further degradation, typically to an acetyl and a succinyl derivative. Each oxygenase reaction is highly exergonic, releasing about 300 kJ/mol reaction (equivalent to 4 or 5 ATP units) as heat, which is the price for the exploitation of a comparably stable group of substrates as energy and carbon source. In all cases of aerobic breakdown of aromatic compounds the primary attack and ring-cleavage step are oxidative reactions of this kind

5. Anaerobic Processes

The fact that oxygen is not available in all environments where hydrocarbons occur (e.g., in deep sediments and in oil reservoirs) has repeatedly evoked the question as to whether or not the biodegradation of hydrocarbons is possible under anoxic conditions, and if so to what extent. Tarvin and Buswell (1934) provided the first evidence that aromatic compounds could be degraded under anoxic conditions, but it was not understood until the late 1980s that novel types of microorganisms were definitively shown to degrade hydrocarbons under strictly anoxic conditions.

The important processes in anaerobic digestion of wastes are hydrolysis, acidogenesis, acetogenesis, and methanogensis, where hydrolysis step is an extra cellular process where the hydrolytic and acidogenic bacteria excrete enzyme to catalyze hydrolysis of complex organic materials into smaller units. The hydrolyzed substrates are then utilized by acidogenic bacteria. Product such as

acetate, hydrogen and carbon dioxide can directly be used by methanogenic bacteria producing methane and carbon dioxide, while other more products such as alcohol and volatile fatty acids are further oxidized by acetogenic bacteria in syntrophic with the methanogens. The whole process is carried out with the help of microorganisms (M. Krishania *et al*., 2013).

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 denitrificansin* (Williams and Evans, 1975). Even though less advanced anaerobic biological process is the most advantageous process due to the low energy requirement, low production of bio-solids and biogas production, which is considered as an innovative energy source (Saghafi *et al.*, 2010). In this pathway phenol is carboxylated in the para position to 4 hydroxybenzoate which is the first step in the anaerobic pathway. The enzyme activity involved in carboxylation has been named 'Phenol Carboxylase', which is located in the cytosol, where it is catalyzes the reversible para-carboxylation of phenol to 4OHBz.

Anaerobic biodegradability is typically evaluated using a biological test, the Biochemical Methane Potential (BMP) test, which produces two values: the BMP value, which is the ultimate amount of methane produced under anaerobic conditions, and the production kinetics. The BMP value can be used as an index of the anaerobic biodegradation potential. The BMP is the experimental value of the maximum quantity of methane produced per gram of volatile solid (VS). The BMP is measured with the BMP test, which consists in a respirometric test, i.e., measuring the methane or biogas produced by a known quantity of waste in a batch in anaerobic conditions (Hansen *et al*., 2004). However this test seems to be time consuming (30 days) using bacterial consortium.

The relationship between anaerobic biodegradability (BD) and BMP is expressed by the following equation by Buffiere *et al*, 2006:

$$BD = \frac{\text{BMP (mlCH4, STP g} - \text{1VS)}}{\text{350xCOD waste(g COD g} - \text{1 VS)}}$$

Where, COD is the Chemical Oxygen Demand and BMP the Biochemical Methane Potential value expressed in the Standard Temperature and Pressure (STP), respectively 273.15 K (0 8C) and 100 kPa (1 atm).

Other factors which determine the rate and efficiency of anaerobic degradation should be analysed by the following criteria in addition to BMP (Cline *et al*., 2010): Specific methane activity (SMA), which measures the rate at which the COD in wastewater is converted to methane. Nutrient deficiency (ND) which is employed to determine whether the wastewater has enough nutrients to allow anaerobic bacteria to grow. Anaerobic toxicity assay (ATA) which indicates whether the wastewater is toxic to anaerobic bacteria. And, cation inhibition (CI) which is used to investigate the effect of cation concentration on the performance of anaerobic bacteria and whether a pH control is required for anaerobic technology. A key step in anaerobic degradation of phenolics is the scission of the benzene ring. The biochemical pathways for phenol degradation based on the work of Chmielowski, Keith and Williams and Evans, 1965 are shown in the figure 1.

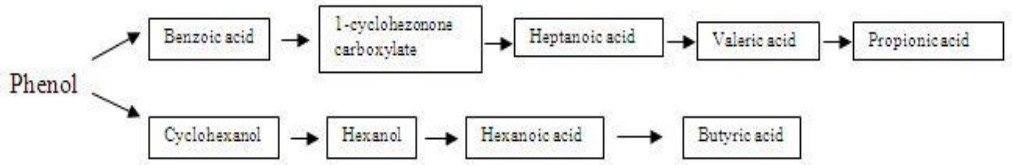


FIGURE 1: biochemical pathways for phenol degradation

The anaerobic pathways have been shown to follow carboxylation reaction in para position to the hydroxyl group of o-cresol results in 3 methyl- 4-hydroxybenzoate (4OHBz) is the first step of its anaerobic degradation. This evidence has been obtained from the studies with one nitrate reducing bacteria, *Thauera aromatica* strain k172, it was isolated from anaerobic sewage sludge, based on its ability to use phenol as its sole source of carbon and energy, and nitrate as the terminal electron acceptor (Tschech and Fuchs, 1987) and *Azoarcus* (although known earlier for its nitrogen fixing capacity but is now more potential towards the degradation of aromatic hydrocarbons including toluene, Zhou *et al.*, 1995), both being the members of β subclass of the proteobacteria. Benzoyl CoA can be regarded as the central intermediate in the anaerobic degradation of many compounds in *T. aromatica* K172 and most likely also in other organisms capable of degrading aromatic compounds (Harwood and Gibson, 1997). Carboxylation is also reported for a denitrifying Paracoccus- like organism (Rudolphi *et al*., 1991) as well as for the methanogenic consortium of Bisaillon *et al*. (1991 b), this methanogenic consortium later showed transformation of a variety of phenolic compounds, including o-cresol, catechol and ortho-halogenated phenols via paracarboxylation followed by dehydroxylation.. Paracarboxylation of m-cresol to 2 methyl-4-hydroxybenzoate has been described for both a sulphate reducing and a methanogenic consortium (Ramanand and Sulflita, 1991; Roberts *et al*., 1990).

Under anaerobic conditions, PCE and TCE can be reductively dehalogenated to the lesser chlorinated.alkenes cis-l,2-, and trans-l,l-dichloroethene (c-DCE, t-DCE), and, at a much slower rate, to monochloroethene (vinyl chloride [VC]) or ethene (Bouwer and McCarty, 1983) while aerobically it follows co metabolism.

6. Phenol carboxylation under denitrifying conditions

Lack and Fuchs (1992,1994) have been able to show that in the whole cells of Strain K172, as well as in cell extracts (U^{14} –C) phenol is first phosphorylated to (U^{14} – C)phenyl phosphate, the phosphoric acid ester of phenol.

Here phenol is apparently phosphorylated via phosphate exchange with phenyl phosphate. The exchange of the phosphate group between phenyl and phenyl phosphate suggests an enzyme- bound phosphate as an intermediate as depicted in figure 2.

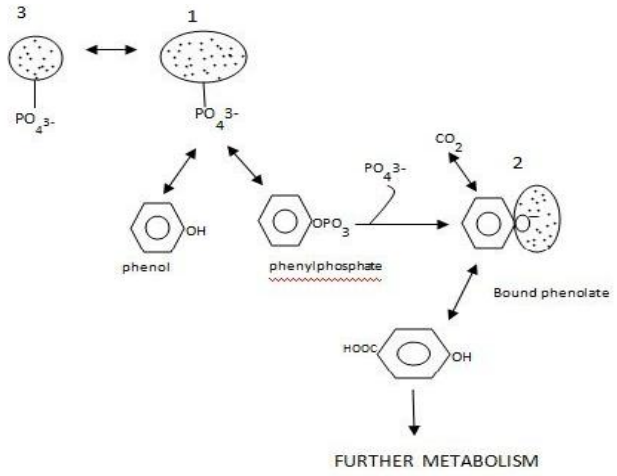
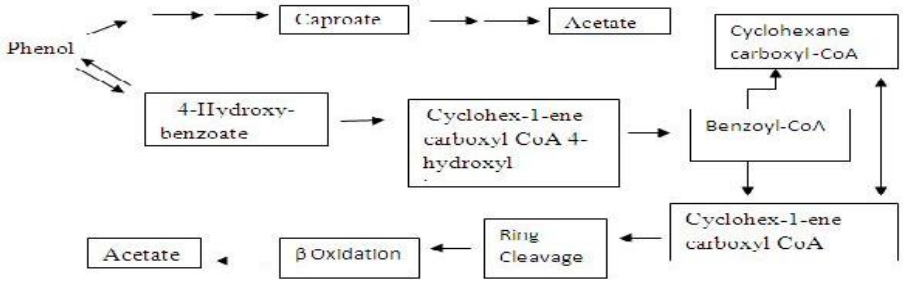


FIGURE 2: Carboxylation of phenol in denitrifying bacteria *Thauera aromatica K172*

Early findings may be explained by the fact that all the reaction in the phenol pathway takes place inside the cell, phenol remains tightly bound to the carboxylase enzyme during carboxylation, and 4OHBz undergoes thioesterification with Co-enzyme A (which helps to retain molecule inside the cell, optimize substrate recognition for the next enzyme(S), and facilitate reductive steps in the pathway because of its electron drawing potential), which is catalysed by 40HBz-CoA ligase (Biegert *et al*, 1993) to form 4OH-benzoyl-Co-A (Tschech and Fuchs,1989). It is further reductively dehydroxylated to benzoyl-CoA by 4OH-benzoyl-CoA reductase (Brackmann and Fuchs, 1993).

7. Phenol carboxylation under methanogenic conditions

Phenol mineralisation under methanogenic conditions is very complex and it proceeds in different pathways. So far, two possible pathways for mineralisation of phenol into methane have been reported; via 4-hydroxybenzoate into benzoyl-CoA or via caproate into acetate (Levén *et al.*, 2010). The degradation of phenol via benzoate is well documented in study of Knoll and Winter (1989). Carboxylation is also a recurring process in methanogenic conditions but only with a small difference that instead of 4OHBz, benzoate is formed as the intermediate (Bechard *et al.*, 1990). The conversion of phenol to methane is the concerted effort of syntrophic bacteria, including fermenters and hydrogen oxidizing methanogens (Szewzyk *et al.*, 1985). Knoll and Winter (1989) have also put forward a phenol degrading obligate syntrophic methanogenic consortium including Methanospirillum hungatei strain, a desulphovibrio sp., and short as well as long rod shaped bacterium. Dehalogenation and degradation of halogenated aromatic compounds by anaerobic bacteria under methanogenic conditions are well established and reviewed by Haggblom *et al.*, 1992. However, methane-producing processes have not been widely used due to low energy recovery from phenol and high operational costs. Cleavage of the aromatic ring followed by oxidation of the fatty acid produces acetic acid. As this acid was not accumulated in the culture as shown by Levén *et al.* (2012), it has to be degraded. Acetotrophic methanogens or acetate-oxidising bacteria in cooperation with hydrogenotrophic methanogens have the ability to degrade acetate. Figure 3 shows two alternative degradation pathways for phenol under methanogenic conditions, either via caproate or via 4-hydroxybenzoate and the benzoyl-CoA pathway



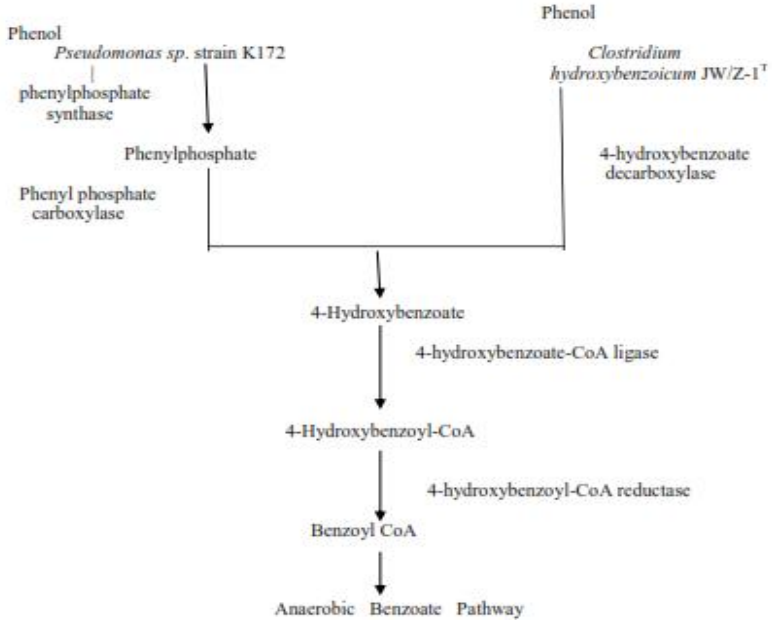
Clostridium hydrobenzoicum (Zhang *et al.*, 1994) and *clostridium Sp*. Strain 6 was also isolated which catalyses methanogenic reaction. Both the organism require amino acids especially arginine and glycine (Zhang *et al.*, 1994) as growth substrates and also for carboxylating activities.An interesting difference between these two strains is that C. hydrobenzoicum can reversibly decarboxylate 4OHBz but is unable to transform phenol to

benzoate, whereas clostridium Sp. Strain 6 can both decarboxylate 4OHBz to phenol and carboxylate phenol to benzoate, presumably with 4OHBz as the intermediate (Li *et al.*, 1997). This conclusion, at least in part may explain the reason why no, or traces of 4OHBz could be detected in the phenol degrading enrichment cultures of other investigators. This finding is also in agreement with the detection of 4OHBz decarboxylating activity in cell free extracts of a defined phenol degrading methanogenic consortium (Gallert and Winter, 1992). The end products of the non-methanogenic anaerobic degradation of phenols are volatile organic acids with other organic intermediates. Although methanogens can be isolated through many techniques the most commonly adapted one is the Hungate's Roll tube technique ((Bryant, 1972; Hungate 1950, 1969; Sowers and Noll, 1995), which includes exclusion of oxygen in the preparation, sterilization of anoxic media, aseptic inoculation and transfer of anaerobic microbes with oxidation reduction potential below (-) 330V. It essentially follows the following criteria:

(a) Use of nature's buffer of carbon dioxide–bicarbonate–carbonate to maintain a pH near neutrality,
(b) Use of cysteine and sodium sulfide as reducing agents, and
(c) Use of resazurine as an O/R indicator that is reddish, when oxidized, and colourless, when reduced at (-) 330 mV.

8. Phenol carboxylation under other environmental conditions

4OHBz has also been reported to be produced by Geobacter metallireducens during phenol degradation under iron reducing conditions (Lovley and Lonergan, 1990). Similarly, Desulfotomaculum sp. Strain also mineralizes 4OHBz and can degrade phenol only if bicarbonate is present in the medium, which indicates that CO_2 may be involved in the degradation of phenol (Kuever *et al.*, 1993). Another phenol- degrading sulfidogen, presumed to be a desulfovibrio sp. was reported to convert phenol to acetate. The phenol degradation profile indicated that the presence of acetate that represents an intermediate of phenol degradation retarded the phenol degradation (Taghreed, 2012). Hydroquinone is degraded by sulfate-reducing and fermenting bacteria. The degradation pathway has been studied with a Desulfococcus strain (Gorny and Schink 1994a) and a fermenting bacterium Syntrophus gentianae. In both cases hydroquinone is first carboxylated to gentisate. Gentisate is activated to gentisyl-CoA through a CoA-ligase reaction. In S. gentianae gentisyl-CoA is reductively dehydroxylated to benzoyl-CoA, which enters the modified benzoyl-CoA pathway. The dehydroxylation of the two hydroxyl groups proceeds in a single step; no monohydroxylated intermediate has been detected or introduced into the reaction.



This pathway was contributed by Jessica Bell, Eva Young, and Stephen Stephens, University of Minnesota. (Md. Mahiudddin, *et al*, 2012)

The new phenol degrading strains are all short, motile, Gram-negative rods.16S ribosomal RNA analysis has shown that these phenol degraders are closely related to each other. Based on 16S rDNA sequence analysis, these isolates included members of the genera Acinetobacter, Alcaligenes, and Rhodococcus. The sequences of the genes encoding the large subunit of a multicomponent phenol hydroxylase (mPH) in these isolates indicated that the mPHs of the gram-negative isolates belonged to a single kinetic class, and that is one with a moderate affinity for phenol; this affinity was consistent with the predicted phenol levels in the phyllosphere (Sandhu *et al.*, 2009). Special consideration should be given to obtain microbes, which are capable of degrading incompatible compounds because biological treatment of wastewaters and contaminated soils always involve degradation of some incompatible mixtures rather than single compounds (Example, methyl and chlorinated phenolic compounds are known to be incompatible substrates and often exists together (Agarry *et al.*, 2008). Lack and Fuchs (1994) proposed that, in the denitrifying Pseudomonas strain K 172, a phenol kinase transfers a phosphate group from an

unknown donor to phenol, forming phenylphosphate which is then further metabolized. He and Wiegel (1995) showed that 4-hydroxybenzoate decarboxylase catalyzes the conversion of phenol to 4-hydroxybenzoate via anaerobic carboxylation in a Clostridium species. The anaerobic degradation is initiated via carboxylation of phenol. The phenol carboxylation proceeds in two steps. The first step involves the phosphorylation of the phenol by the addition of a phosphate group from an unknown phosphoryl donor catalysed by a phosphorylated enzyme called phenyl phosphate synthase (kinase) to form phenyl phosphate as the first intermediate. The second step involves the carboxylation of phenyl phosphate catalysed by a Mn^{2+} requiring enzyme, phenyl phosphate carboxylase to form 4-hydroxybenzoate. As already discussed paracarboxylation is followed by dehydroxylation. It is known that decarboxylation reactions can create proton gradients across the cell membranes. These proton gradients, or 'proton motive forces', can generate electrochemical energy (Konings *et al.*, 1995; Mitchell, 1968). Gallert and Winter (1994) have shown that the intercellular ATP levels in their defined methanogenic consortium increased after decarboxylation of 4OHBz to phenol.Consequently, cell yields on 4OHBz were significantly higher than with phenol as the substrate. Additionally, a proton ionophore (carbonyl cyanide m-cholophenyl hydrozone; CCCP) or an H^+ - ATPase inhibitor (Dicyclohexyl carbodiimide; DCCD) both decreased ATP levels. These results were taken as the evidence that decarboxylation of 4OHBz generates a proton gradient across the membrane. Evans (1977) summarized probable pathways for the anaerobic biodegradation of aromatic compounds. He established that the conversion of aromatic compounds to CH_4, and CO_2 is accomplished first by the hydrogenation of the benzene nucleus with the formation of some alicyclic compounds as intermediate products; the alicyclic rings are then cleaved to form aliphatic acids and subsequently, volatile organic acids (VOA). Finally, VOA is converted to suitable substrates (acetate, hydrogen, formate) for methanogens to complete the process. Removal of intermediates by methanogens provides thermo-dynamically favorable conditions for the degradation of many aromatic compounds. Yi-Tin Wang *et al.* (1989). Recently, Sheridan *et al.*, 1985 and Dwyer *et al*., 1986, 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*.

(1) $C_6H_6 + 5H_2O \longrightarrow 3CH_3COOH + 2H_2$

(2) $3CH_3COOH \longrightarrow 3CH_4 + 3C0_2$

(3) $2H_2 + 0.5C0_2 \longrightarrow 0.5CH_4 + H_2O$

Sum $C_6H_6O + 4H_2O \longrightarrow 3.5CH_4 + 2.5C0_2$

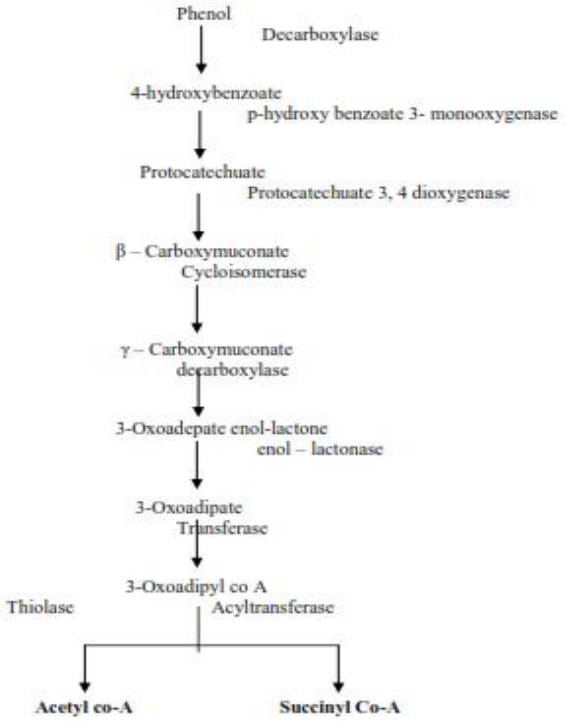
Detailed analysis of anaerobic processes reveals the identification of three key intermediates by which mononuclear aromatic compounds are channelled, *i.e*., benzoyl-CoA, resorcinol, and phloroglucinol (Evans and Fuchs 1988; Fuchs *et al*. 1994; Heider and Fuchs 1997., Schink *et al*. 1992). The common feature of the three pathways is that the aromatic nucleus is destabilized via a reductive rather than an oxidative attack. The benzoyl-CoA pathway appears to be the most important one because a broad variety of compounds enter this path, including phenol, various hydroxybenzoates, phenylacetate, aniline, certain cresols and even the hydrocarbon toluene (Harwood *et al*. 1999; Heider and Fuchs 1997; Schink *et al*. 1992). Benzoyl-CoA is formed either through a variety of channeling reactions (*e.g*., removal of substituents such as hydroxy groups) which are carried out at the CoA-ester level or directly from benzoate and HSCoA in an AMP-releasing ligase reaction. Once benzoyl-CoA is formed, the stability of the aromatic ring structure is overcome by a reductive step, as shown with the nitrate-reducing bacterium *Thauera aromatica*. Two single electrons and protons are introduced, probably through a radical intermediate, to form cyclohexadiene carboxyl-CoA as first identifiable product (Boll and Fuchs 1995; Koch *et al*., 1993). Since the reduction in the benzene ring to a cyclohexadiene derivative is an endergonic reaction with its physiological electron donor ferredoxin (E0'p–450 mV; Boll and Fuchs 1998), it requires the investment of energy in the form of ATP. The present concept is that 2 ATP are hydrolyzed to ADP+Pi, probably one with each electron introduced (Boll *et al*. 1997). Thus the benzoyl-CoA pathway as described here involves a rather high energy input for substrate-activation and dearomatization. Nitrate-reducing bacteria can recover this energy investment through the further breakdown of the C7-dicarboxylic acid derivative produced upon ring cleavage, via b-oxidation to three acetyl-CoA residues which are finally oxidized in the citric acid cycle. Resorcinol and phloroglucinol both carry hydroxyl groups in meta position to each other, which allows tautomerization to the enol form. This generates an isolated double bond which can be easily reduced. An entirely different strategy is taken in the anaerobic degradation of resorcinol and its carboxylated derivatives. The two hydroxyl groups in resorcinol are in positions to allow tautomerization to an unsaturated cyclohexenedione derivative with an isolated double bond. Cell-free extracts of a fermenting Clostridium strain convert resorcinol to dihydroresorcinol (Kluge *et al*., 1990; Tschech and Schink 1985) to form cyclohexanedione, which is further hydrolyzed to 5-oxohexanoate, probably by a nucleophilic attack on one of the carbonyl carbon atoms. The resorcinol reductase of this bacterium consists of subunits of 49.5 kDa and contains flavin adenine dinucleotide, but iron sulfur centers have not been detected (Schüler, 1997). These decarboxylations are chemically easy because in these cases the carboxylic group is located in ortho- or para-position to hydroxyl groups. No resorcinol-reducing activity can be identified in cultures of nitrate-reducing bacteria growing with resorcinol as sole substrate as it follows an entirely different strategy. (Gorny *et al*., 1992; Kluge *et al*., 1990). *Azoarcus anaerobius* does not cleave the ring hydrolytically, but the resorcinol ring is destabilized by the introduction of a further hydroxyl group to form HHQ. The new pathway of resorcinol degradation through hydroxylation to HHQ also opened a solution for anaerobic degradation of a-resorcylate (3,5-dihydroxybenzoate).The enzyme activity involved is membrane bound, and the hydroxylation can be coupled to nitrate reduction to nitrite or to reduction of other electron acceptors with an E0' higher than +100 mV. The reaction

leads, among others, to an acetate and a succinate residue, suggesting that the HHQ intermediate is cleaved between the carbon atoms 1 and 2 and 3 and 4 and, as far as is known. HHQ is oxidized to hydroxy benzoquinone in a further oxidation step with a standard redox potential of c180 mV (Philipp and Schink 1998), which can explain why this strategy is followed only by nitrate-reducing bacteria not by sulphate reducing or fermenting bacteria but a further alternative of HHQ degradation was found with the sulfate- reducing bacterium *Desulfovibrio inopinatus*. This bacterium metabolizes HHQ according to the following equation according to Schink B. *et al*, 2000:

$$C_6H_6O_3 + H_2O + SO_4^{2-} \longrightarrow 2CH_3COO^- + 2CO_2 + HS^- + H^+$$

The cleavage products found can easily be oxidized to CO_2, with nitrate as electron acceptor. Among the three trihydroxybenzene isomers, pyrogallol and phloroglucinol are degraded quickly by fermenting bacteria and were the first aromatic compounds to be degraded by fermentation in pure culture (Schink and Pfennig 1982). Phloroglucinol degradation has been studied in detail with *Eubacterium oxidoreducens* and *Pelobacter acidigallici*. Phloroglucinol is reduced by an NADPH-dependent reductase to dihydrophloroglucinol. The third trihydroxybenzene isomer, HHQ, is converted by the fermenting bacterium *Pelobacter massiliensis* to three acetate as well (Schnell *et al*. 1991), indicating that this pathway also leads through phloroglucinol. The isomerization to phloroglucinol requires three subsequent transhydroxylation reactions analogous to the pyrogallol-phloroglucinol transhydroxylation, and indeed phloroglucinol is the final aromatic compound that is reduced and cleaved hydrolytically (Brune *et al*., 1992).

Flow chart of anaerobic degradation pathway for phenol. (V. SRIDEVI *et al*., 2012)



9. Advanced technologies in Phenol degradation

Microbial fuel cell (MFC) has gained a great attention attributable to its ability in generating electricity directly from and potentially enhancing biodegradation of contaminants. In the MFC, electrons released from the substrate oxidation in the anode are transferred via the external circuit to the cathode, where the electrons are eventually consumed by the terminal electron acceptors. The terminal electron acceptors can be easily replaced or even non-exhausted (*e.g.*, using oxygen in ambient air as the electron acceptor). When phenol served as the sole fuel, the peak voltage output in MFC was obtained when 90% of phenol was depleted. Co-occurring with electricity generation, the degradation efficiencies of phenol in all the MFCs reached above 95% within 60 h. The results indicate that the MFC can enhance biodegradation of recalcitrant contaminants such as phenol in practical

applications. The degradation rates of phenol in the MFC increased about 15% as compared to the open-circuit control. (Luo *et al.*, 2008). In a few cases, some biorefractory organics, such as cellulose and petroleum contaminants, were also used as the fuel in MFCs. Water desalination may also be feasible with energy derived from wastewater in a novel microbial fuel cell design. Now a days “electro-bioremediation” (a hybrid technology of electrokinetics and bioremediation) for treatment of soil pollutants by utilization of a low-voltage electric field is fast growing. Contrary to an MFC, operation of the bio-electrochemical cell (BEC) is maintained under constant voltage and not under constant resistance. The combination of electrokinetic and bioremediation

technologies in the BEC enable phenol degradation by a monoculture of *Cupriavidus basilensis* bacterial cells even under conditions of limited dissolved oxygen. Furthermore, the bacterial cells were electro-active and produced electricity (Hen *et al*., 2013). The phenomenon of better bacterial cell growth in a poised potential BEC (with bacterial cells) compared to a control BEC using a monoculture of *P. putida* grown with toluene as the sole carbon source was recently reported (Friman *et al*., 2012) which was supported by the study of Hen *et al*, 2013 where, 80% of phenolic degradation was observed in poised potential BEC when fed with phenol concentration of 100, 200 and 400 mg/l. However, many studies agree that mixed microbial populations seem to perform better in MFCs when complex organics are used as the fuel. Electro-osmosis has been shown to efficiently remove water dissolved phenol, o-nitrophenol, hexachlorobenzene, benzene, toluene, ethylene and xylene, hexane, isooctane, and trichloroethylene from clay (Wick *et al*., 2007). Electrodes can supply electrons to support the respiration of some microorganisms Lovley *et al.* (2010) or can accept electrons, serving as an electron acceptor to support anaerobic oxidation of organic compounds or inorganic electron donors such as hydrogen and elemental sulphur (Pant D, 2010). Electron flow gains significance because of the ability of microorganisms to consume or produce electrical current has potential practical applications in the environmental and bioenergy fields. Water is abundant and ubiquitous, mak- ing it an ideal electron donor for many perceived cathode applications Lovley *et al*. (2010). The possibility of using solar technology to sustainably generate the electricity necessary to supply the electrons for such groundwater bioremediation efforts is particularly attractive .One of the most suitable areas for the practical application is bioremediation of aquatic sediments and groundwater. Inexpensive but durable graphite electrodes deployed in sediments not only serve as a low-maintenance, long-lived, desirable electron acceptor for anaerobic respiration, but also can adsorb contaminants from the surrounding sediment, co-localizing the contaminants, contaminant-degrading microorganisms, and an electron acceptor in the same location as suggested by Zhang *et al*(2010). Electrons can be directly supplied for the electrode- microbe interaction to utilize them for many bio-catalyst processes or else they can also be supplied indirectly via the production of hydrogen gas or the reduction of electron shuttle molecules (Derek *et al*, 2011). Near the earth surface, the sun produces 0.2-0.3 mol photons $m^{-}2$ $h^{-}1$ in the range 300-400 nm with a typical UV flux of 20-30 W $m^{-}2$ (S. Ahmed *et al*., 2011). This suggests using sunlight as an economically and ecologically sensible light source. As a result, development of an efficient photocatalytic water purification process for large-scale applications has received substantial interest, but still it remains a challenge. So, solar and other forms of renewable electrical energy can be used to provide electrons extracted from water to microorganisms on electrodes at suitably low potentials for a number of groundwater bioremediation applications as well as for the production of fuels and other organic compounds from carbon dioxide.

The degree of electrostatic attraction or repulsion between the photocatalyst's surfaces and the ionic forms of organic molecule can vary with the change in solution pH, which can result in enhancement or inhibition of the degradation of organic pollutants in the presence of TiO2. The pH of the solution affects the formation of hydroxyl radicals by the reaction between hydroxide ions and photo-induced holes on the TiO2 surface (S. Ahmed *et al*., 2011). The positive holes are considered as the major oxidation steps at low pH, whereas hydroxyl radicals are considered as the predominant species at neutral or high pH levels (Shifu , Gengyu, 2005). Valenzuela *et al*. (2002) observed the following pattern of photocatalytic activity TiO_2 P25 > $ZnFe_2O_4$ > ZnO > Fe_2O_3 for the degradation of phenol and the photocatalytic degradation of phenol and ortho substituted phenolic compounds was shown to follow the order: Guaiacol > 2-chlorophenol = Phenol > Catechol (Peiro *et al*., 2001). Sonoelectrochemistry (a technology utilizing electrochemistry and ultrasound) has the great advantage of being carried out under very mild conditions. The efficiency of sonochemical process for phenolic compound degradation has been previously demonstrated (Gogate, 2008). His study summarizes that the synergistic effect of combining ozonation with ultrasonic irradiation is observed only when the free radical attack is the controlling mechanism and the rate of generation of free radicals due to ultrasonic action alone is somewhat lower. The rate of sonolytic ozonation will be higher at lower concentration of the pollutant and hence dilution of the effluent stream may be considered as a pretreatment scheme. The basic reaction mechanism for both ultrasound initiated degradation process as well as photocatalytic oxidation (either using UV light or solar energy) is the generation of free radicals and subsequent attack by these on the pollutant organic species and if UV and ultrasound are operated in combination then the chances of generating free radicals are even more. For majority of the phenolic contaminants, the reaction takes place usually in the liquid bulk or gas-liquid interface and hence the decrease in the rates of degradation process will be significant.

A detailed analysis of the reaction products indicated that presence of TiO_2 resulted in formation of higher concentrations of low molecular weight products. This is especially important when the ultrasonic irradiation has to be used in combination with biological oxidation as a treatment scheme. The main sonochemical mechanism of phenolic compound degradation is carried out by hydroxyl radical oxidation. The combination of ultrasound (US) and ultraviolet (UV) irradiation has received great attention since last decade. Tezcanli–Güyer and Ince, 2004 put into evidence the synergic effects of this combined process where they studied the efficiency of textile dye by using UV and US radiations individually and also using a combination of UV/US irradiations. The relatively high performance of the US/UV process was mainly attributed to the photo-dissociation of H_2O_2 formed during water sonication, which increases the hydroxyl radical production. The increase of the degradation rate observed in several studies may be the result of four processes: direct photolysis of the organic solute, photodissociation of oxygen peroxide, enhanced transfer of ozone formed in the gas phase by ultrasonic fountain, and then indirect photochemistry induced by the nitrogen oxides formed

during ultrasonic irradiation of water saturated with air or nitrogen (F. Zaviska *et al.*, 2014). Aeration has also been found to enhance the rates of ultrasonic degradation of organic pollutants possibly due to the availability of oxygen.

It appeared that nitrate and nitrite ions tend to inhibit the sonochemical degradation of phenol. This can be partially explained by the fact that nitrite ions are known to scavenge hydroxyl radical with k = 1 X 1010 sec-1 (G.V. Buxton *et al*, 1988). This observation was supported by experiment conducted by (F. Zaviska *et al.*, 2014) where, US and US/UV effects was analysed and the apparent reaction constant (k) of phenol degradation in presence of nitrate were significantly lower when compared to the rates in the absence of nitrate. Thus, nitrate photolysis lowers phenol photolysis because nitrate act as UV filter and thus inhibits organic compound photolysis. Generally, sono-degradation of substituted phenols was easier than phenol in a mixture, but there was an exception in the combination of phenol and p-nitrophenol that the degradation of phenol was faster than substituted compound probably due to the presence of nitro group as discussed above.

More recently, in situ bioaugmentation approach for phenol removal from soil has been addressed (Wang *et al*, 2011). The design of an in situ remediation application requires ascertaining the relative impact of factors such as properties of the contaminant, the soil environment and soil fraction composition and reactivity on biodegradation rates to secure successful in field implementation. The biodegradation of phenol using different types of microbial cultures, especially some plant associated bacteria, has attracted the attention of many researchers during the past two decades. Horizontal gene transfer has been shown to be the key mechanism in the evolution of many haloalkane-degrading bacteria. This genetically modified strain should be capable of playing an important role in environmental remediation and various agronomic applications. The naphthalene-degrading bacteria have been shown to arise from natural horizontal transfer of a naphthalene dioxygenase gene, which may play a significant role in the acclimation of microbial communities to pollutants. Some *Sphingomonads* strains have acquired chlorophenol-degrading abilities from natural horizontal transfer of the pcpB gene encoding for pentachlorophenol-4-monooxygenase (L. Yang *et al*., 2011).

Owing to the global energy crisis efforts are made to develop bio-oils (pyrolysis oil) to substitute heavy petroleum products, high value-added chemicals and other products, but their composition show that bio-oils are now a major contributors of the phenolics (a mixture of phenol, guaiacol and other substituted phenol compositions). Bio-oils are unstable in nature due to the negative properties of chemical constituent's and also due to high oxygen content. This feature makes them susceptible to react freely with any chemical compounds present in nature and gets converted to more complex organic compounds which are more difficult to break down.Catalytic hydrodeoxygenation (HDO) of bio-oil involves the presence of catalyst and hydrogen at moderate temperature (300–600 ^{0}C). The oxygen is removed in the form of water (Bu *et al*., 2012).

10. Phenol kinetics

Information about the kinetics of phenol biodegradation is necessary for optimal design and operation of biological treatment systems. As the inhibitory nature of the phenol is well known, many substrate inhibition models are in existence now-a- days to describe the dynamics of pure and mixed culture microbial growth on phenol . However, despite of model's empirical nature, these substrate inhibition models are able to accurately describe experimental phenol biodegradation data, thereby providing a convenient means of modelling phenol biodegradation. Of the various substrate inhibition models, the Andrews equation (equation 1) has been used extensively to describe phenol biodegradation (Livingstone and Chase, 1989). The Andrews equation relates microbial specific growth rate (μ) and limiting substrate concentration (S) as (Andrews, 1968).

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2/Ki} \qquad \text{------- Equation1}$$

Where μ = microbial specific growth rate, h^{-1}; Ks = saturation constant, g/L; and K_i = inhibition constant, g/L.

Cases where different substrate inhibition models accurately describe mixed culture phenol degradation and also when no statistically significant difference is observed among the models the generalized substrate inhibition model (GSIM) of Tan *et al*. (1996) can be used, which describes substrate inhibition of microbial growth using a statistical thermodynamics approach. The GSIM describes substrate inhibition of microbial growth, assuming that each microbial cell consists of a number of basic, identical functional units, with each unit composed of n binding sites for substrate molecules and m inhibition sites. It relates microbial specific growth rate and substrate concentration as:

$$\mu = \frac{\alpha_1 [S] + \alpha_2 [S]^2 + \ldots + \alpha_{n-m} [S]^{n-m}}{\beta_0 + \beta_1 [S] + \beta_2 [S]^2 + \ldots + \beta_n [S]^n} \qquad \text{----------Equation 2}$$

Where S = substrate concentration, and αi and βi = constants (functions of binding energy and initial reaction rates, respectively).

A substrate inhibition model of the form (as in equation 3) has been found to best represent specific phenol utilization. This model indicates a maximum specific utilization rate occurring at phenol levels of 686mg/l.

$$V = 0.08 \Big/ \left(1 + (700/S) + (S/966)^4\right)$$

Monod equation is a simple model which predicts cell growth on a non-inhibitory growth-limiting substrate as in equation 4:

$$\mu = \frac{\mu_m S}{K_s + S}$$

Where μ represents the specific growth rate (1/h), S the substrate concentration (mg/l), μm is the maximum growth rate (1/h), Ks is the half-saturation substrate constant (mg/l). In order to consider toxicity effect of the substrate, Haldane or Andrew's equation, a modification

Monod model is used as given in equation 5. The experimental specific growth rates were obtained on the basis of Malthus law for various phenol concentrations:

$$\mu = \frac{dX}{Xdt} \quad \text{---------- Equation 5}$$

The parameter X stands for concentrations of biomass. It can be defined on the basis of either cell dry weight (mg/l) or optical density of the culture at a specific wavelength. According to the substrate-inhibition model used, based on the Haldane equation and valid for substrate concentrations less than 400 mg l–1, the maximum specific growth rate (μmax) and the Monod constant (K_s) increased with increasing temperature. The simultaneous increase of the inhibition constant (K_i) indicated an increased degree of inhibition at low temperatures. A novel, integrated 'Best Equation' describing microbial growth influenced by substrate availability and inhibition was recently presented (Hanzel *et al*., 2012) almost 60 years after the first one had appeared.

According to Taghreed *et al*, 2012, phenol concentrations with the value of 50-400 mg/l did not show any obvious inhibition effect on growth of microorganism. The media with phenol concentration of 400mg/l required more time to grow than the media with low phenol concentrations. This behaviour was due to substrate inhibition. The phenol substrate was mainly consumed for assimilation into biomass and energy for cell growth and maintenance. (Wang *et al*, 2007). However at high substrate concentration, the inhibition effects on cell growth were stronger than low substrate concentration and also more energy was required to maintain the cell viability, but may not be enough to maintain the cell viability. The effect of a toxic compound on a treatment process is quantified in terms of the inhibition coefficient, Ki. It should be noted that when Ki is very large the Haldane equation simplifies to the Monod equation (implies that the culture is less sensitive to substrate inhibition). So, low values of Ki showthat the inhibition effect can be observed at low phenol concentration.

The apparent KS value is of practical importance because a bacterium expressing activity with a lower apparent KS value can efficiently remove the pollutant down to lower concentration. At higher substrate concentrations, S_KS, the Haldane equation becomes:

$$\mu = \frac{\mu m}{1 + (S/Ki)}$$

CONCLUSION

The continuous and essential cycling of carbon in the biosphere depends upon a balance between the synthesis and degradation of organic carbon. Many aromatic hydrocarbon derivatives are industrial by-products and are considered to be not readily biodegradable. These refractory compounds, along with some naturally refractory polymers, can end up as waste material and eventually accumulate in the environment. This accumulation threatens the normal balance in the carbon cycle. There is need, therefore, to reduce the amount of refractory material in wastes which need disposal, which signifies the role of biodegradation of many complex aromatic organic compounds in either aerobic conditions or in anaerobic conditions. As priority pollutants, phenolic compounds must be eliminated, within the frame of sustainable development to preserve the environmental quality. It involves the breakdown of these organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic), or CH_4 (anaerobic).

Saturated and aromatic hydrocarbons are wide-spread in our environment. Phenols are common starting materials and often waste by-products in the manufacture of industrial and agricultural products. These compounds exhibit low chemical reactivity and for many decades were thought to undergo biodegradation only in the presence of free oxygen. During the past decade, however, an increasing number of microorganisms have been detected that degrade hydrocarbons under strictly anoxic conditions. Now, significant advances have been made in the understanding of the anaerobic biodegradability of monoaromatic hydrocarbons. It is now known that compounds such as benzene, toluene, ethyl benzene, and all three xylene isomers can be biodegraded in the absence of oxygen by a broad diversity of organisms. These compounds have been shown to serve as carbon and energy sources for bacteria growing phototrophically, or respiratorily with nitrate, manganese, ferric iron, sulfate, or carbon dioxide as the sole electron acceptor. Oxidation of organic compounds coupled to the reduction of sulphate to sulphide accounts for more than 50% of carbon mineralization in marine sediments. Glucose addition up to a specific low concentration could improve the degradation rate, but impeded the degradation process at higher concentrations. It is understood that fermentive or acetogenic bacteria first transform the aromatic compounds to methanogenic precursors such as acetate and hydrogen, methanogenic bacteria then converts these substrates to methane and CO2 which requires a consortium because of the limited range of methanogenic bacteria. Immobilization in spite of being advantageous owing to its easy transportation and storage, it also raises concerns due to its efficiency deterioration when environmental alterations occur. Toxic and biorefractory organics, which were found frequently in the wastewater, have a great influence on the wastewater treatment and should be concerned in the related MFC research. Combining the benefits of power generation in offsetting the treatment cost, the MFC may offer a new technique in enhancing biodegradation of recalcitrant contaminants such as phenol in practical applications. However, the development of MFCs using recalcitrant contaminants as fuels is still in its infancy and warrants further research. It seems likely that any organic contaminants that microbes have been shown to anaerobically oxidize with other electron acceptors can be oxidized with electron transfer to an electrode. The photocatalytic degradation of various toxic organic compounds has been proposed as a viable process to detoxify aquatic solutions. Hence, it can be concluded that microorganisms have developed certain in-built mechanisms based on the availability of oxygen and many other external environmental factors to use phenol as its sole source of carbon required for its growth. Heterogeneous photocatalytic oxidation (HPO) process employing catalyst such as TiO_2, ZnO, etc., and UV light has demonstrated promising results for the degradation of persistent organic pollutants, and producing more biologically degradable and less toxic substances.

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REFERENCES

Abdullah-Al-Mahin, M. Chowdhury A. Z. and Fakhruddin A. N. M. (2010) RW 01 Phenol biodegradation by *Pseudomonas putida* CP1 and A (a), Proceedings of International Conference on Environmental Aspects of Bangladesh (ICEAB10), Japan.

Agarry, S. E., Durojaiye, A. O. and Solomon, B. O (2008) Microbial degradation of phenols: A review. Int. Journal of Environ.Poll. 32, 12–28.

Aksu Z. and Gonen F (2006) Binary biosorption of phenol and chromium (VI) onto immobilized activated sludge in a packed bed: Prediction of kinetic parameters and breakthrough curves, Separation Purification Technology 49 (3), 205 – 216,

Aksu, Z., F. G¨onen, (2004) Biosorption of phenol by immobilized activated sludge in a continuous packed bed: prediction of breakthrough curves,Process Biochem. 39 (5): 599–613.

Alexander ,M. (1999) Biodegradation and bioremediation, 2nd edn. Academic Press, London.

Alexander B., Leach S., Ingledew W.J. (1987) The relationship between chemiosmotic parameters and sensitivity to anions and organic acids in the acidophile *Thiobacillus ferrooxidans*. Journal of General Microbiology 133, 1171–1179.

Alexander, M. (1965) Biodegradation: Problems of molecular recalcitrance and microbial infallibility. Advanced Applied Microbiology 7, 35-80.

Andrews, J.F. (1968) A Mathematical Model for the Continuous Culture of Microorganisms Utilizing Inhibitory Substrates. Biotechnology and. Bioengineering 10, 707.

Annadurai, G., Ling, L. Y., Lee, J. F (2008) Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. Journal of Hazardous Material 151,171–178.

Armenante, P.M., Fabio Fava. & David Kafkewitz (1995) Effect of Yeast extract on growth kinetics during aerobic biodegradation of chlorobenzoic acids. Biotechnology. and Bioengineering 47, 227-233.

Astley, S.B., (2003) Dietary antioxidants past, present and future. Trends in Food Science Technology 14, 93-98.

ATSDR (1998) Toxicological profile for phenol. U.S Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, Division of Toxicology. Toxicology Information Branch. Atanta. Georgia.

Aziz, A.P.K. (1978) Ecology of the retting grounds in the backwater system of Kerala. Ph.D thesis submitted to University of Kerala. Thiruvananthapuram.

Bak, F., Widdel,F.(1986) Anaerobic Degradation of Phenol and Phenol Derivatives by *Desulfobacterium phenolicum sp*. Archies of Microbiology 146, 177-180.

Bakker,G (1977) Anaerobic Degradation of Aromatic Compounds in the Presence of Nitrate. FEMS Lett. 1,103-108.

Basha Khazi Mahammedilyas, Aravindan Rajendran, and Viruthagiri Thangavelu (2010) Recent advances in the Biodegradation of Phenol: A review, Asian Journal of Experimental Biology. Science Vol 1 (2), 219-234.

Bechard, G.,. Bisaillon J.-G, Beaudet R. and Sylvestre M. (1990) Degradation of Phenol by a Bacterial Consortium under Methanogenic Conditions. Canadian journal of Microbiology 36, 573-578.

Biegert, T., Altenschmidt U.,. Eckerskorn, C., and. Fuchs, G (1993) Enzymes of Anaerobic Metabolism of Phenolic Compounds. 4-Hydroxybenzoate-CoA Ligase from a Denitrifying Pseudomonas Species. Europian Journal of Biochemistry 213,555-561.

Boll M, Albracht SSP, Fuchs G (1997) Benzoyl-CoA reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. A study of adenosintriphosphatase activity, ATP stoichiometry of the reaction and EPR properties of the enzyme. Europian Journal of Biochemistry 244, 840–851.

Boll M, Fuchs G (1995) Benzoyl-CoA reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica strain K172*. Europian Journal of Biochemistry 234, 921–933.

Bouwer, E.J.,. McCarty P.L. (1983) Transformation of land 2-Carbon Halogenated Aliphatic Organic Compounds Under Methanogenic Conditions. Applied Environmental Microbiology 45, 1286-1294.

Brickman, R., Fuchs ,G. (1993) Enzymes of Anaerobic Metabolism of Phenolic Compounds. 4-HydroxybenzoylCoA Reductase (Dehydroxylating) from a Denitrifying *Pseudomonas* Species. European Journal of Biochemistry 213,563-571.

Bronner, G.,Goss, K.-U(2011b) Sorption of organic chemicals to soil organic matter: influence of soil variability and pH dependence. Environmental Science and Technology 45, 1307–1312.

Brune A, Schink B (1992) Phloroglucinol pathway in the strictly anaerobic *Pelobacter acidigallici*: fermentation of trihydroxybenzenes to acetate via triacetic acid. Archies of Microbiology 157, 417–424.

Bryant, M. P (1972) Commentary on the Hungate technique for culture of anaerobic bacteria. American Journal of Clinical Nutrition 25, 1856–1859.

Bu Quan , Hanwu Lei , Alan H. Zacher , Lu Wanga, Shoujie Ren , Jing Liang , Yi Wei , Yupeng Liu ,Juming Tang , Qin Zhang , Roger Ruan (2012) A review of catalytic hydrodeoxygenation of lignin-derived phenols from biomass pyrolysis. Bioresource Technology 124, 470–477.

Buffiere P, Loisel D, Bernet N, Delgenes JP (2006) Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Science Technology 53, 233–41.

Chakraborty R., J. D. Coates (2004) Anaerobic degradation of monoaromatic hydrocarbons, Applied microbiology and biotechnology, Volume 64(4), 437-446.

Chakraborty.R, Susan M. O'Connor, Emily Chan, John D Coates (2005) Anaerobic Degradation of Benzene, Toluene, Ethylbenzene, and Xylene Compounds by *Dechloromonas Strain RCB*, Applied and Environmental Microbiology Vol.71 (12).8649–8655.

Chitra(1995) Studies on biodegradation of phenolic compounds by Pseudomonas pictorum. PhD thesis CLRI.University of Madras, Chennai-25.

Chmielowski, L, A. Grossman, and Labazek, S. (1965) Biochemical Degradation of Some Phenols During Methane Formation. Zesz: Nauk. Poly tech. Slask. Inz: San it. 8:97122.

Cline Taylor , Nathan Thomas , Logan Shumway , Irene Yeung , Conly . Hansen ,Lee D. Hansen , Jaron C. Hansen (2010) Method for evaluating anaerobic digester performance .Bioresource Technology 101, 8623–8626.

Dagley S (1971) Catabolism of aromatic compounds by micro-organisms. Advanced Microbial Physiology 6:1–46.

Das Debadatta and Kaur Rajdeep, Biodegradation of phenol by various indigenous microorganisms (2012) Journal of Environmental Research And Development Vol. 7 No. 2A.

Derek R Lovley and Kelly P Nevin, (2011), A shift in the current: New applications and concepts for microbe-electrode electron exchange, COBIOT-854.

Djokic, L., Narancic, T., Nikodinovic-Runic, J., Savic, M., Vasiljevic, B (2011) Isolation and characterization of four novel Gram-positive bacteria associated with the rhizosphere of two endemorelict plants capable of degrading broad range of aromatic substrates. Appl. Microbiol. Biotechnol. 91, 1227–1238.

Dwyer, D. F., Krumme M. L., Boyd, S. A, and Tiedje, J. M. (1986) Applied Environmental Microbiology, 52, 345.

EPA (1979). Phenol ambient water quality criteria. Office of the planning and standards. Environ. Protect. Agency, Washington, DC, BB.296-786.

Evans , W. C. (1977), Nature, 270, 17.

Evans, W.C.(1947) Oxidation of phenol and benzoic acid by some soil bacteria. J. Biol. Chem., 41:373-382.

Fang, H.H.P. and Chan, O.C. (1997)'Toxicity of phenol towards anaerobic biogranules [J]',Wat. Res., Vol. 31, pp.2229–2242.

Fedorak, P.M. and S.E. Hrudey (1988) Anaerobic Degradation of Phenolic Compounds with Application to Treatment of Industrial Waste Waters. In: Biotreatment Systems,Vol. 1., pp. 170-212. (D.L. Wise, Ed.). CRC Press: Boca Raton, FL

François Zaviska, Patrick Drogui , Errachid M. El Hachemi, Emmanuel Naffrechoux (2014) Effect of nitrate ions on the efficiency of sonophotochemical phenol degradation Ultrasonics Sonochemistry 21, 69–75.

Friman, H., Schechter, A., Nitzan, Y., Cahan, R (2012) Effect of external voltage on *Pseudomonas putida* F1 in bio electrochemical cell using toluene as a sole carbon and energy source. Microbiology, 158.

Fumihisa Kobayashi, Teruya Maki, Yoshitoshi Nakamura (2012) Biodegradation of phenol in seawater using bacteria isolated from the intestinal contents of marine creatures, International Biodeterioration & Biodegradation 69,113-118.

G.V. Buxton, C.L. Greenstock, W. Phillip Helman, A.B. Ross (1988) Phys. Chem. Ref. Data 17 513.

Gallert, C. and J. Winter (1994) Anaerobic Degradation of 4-Hydroxybenzoate: Reductive Dehydroxylation of 4-Hydroxybenzoyl-Coa and ATPFormation During 4-Hydroxybenzoate Decarboxylation by the Stable, Strictly Anaerobic Consortium. Applied Microbiology and Biotechnology 42:408-414.

Gaudy AF,Jr & Gaudy,E.T (1980), Microbiology for environmental Scientists and engineers.Mc Graw Hill Book Co., Newyork.

Ghasemzadeh Ali and Ghasemzadeh Neda,(2011),Flavonoids and phenolic acids: Role and biochemicalactivity in plants and human, journal of Medicinal Plants Research Vol. 5(31), pp. 6697-6703.

Gieg, L.M, Kolhatkar R.V, McInerney M.J, Tanner RS, Harris SH Jr, Sublette KL, Suflita JM (1999) Intrinsic bioremediation of petroleum hydrocarbons in a gas condensate-contaminated aquifer. Environment Science and Technology 33:2550–2560.

Gladyshev, M. I., Sashchik, N. N., Kalachova, G. S., and Shchur, L. A. (1998). The effect of algal blooms on the disappearance of phenol in a small forest pond. Water Res. 32, 2769–2775.

Gogate,P.R., (2008) Treatment of wastewater streams containing phenolic compounds using hybrid techniques based on cavitation: a review of the current status and the way forward, Ultrason. Sonochem. 15 (1–15)

Gorny, N., Wahl G, Brune A, Schink B (1992) A strictly anaerobic nitrate-reducing bacterium growing with resorcinol and other aromatic compounds. Arch Microbiol 158:48–53.

Hadj Benhebal , Messaoud Chaib , Thierry Salmon , Je´re´my Geens , Ange´lique Leonard , Ste´phanie D. Lambert , Michel Crine , Benoıˆt Heinrichs (2013) Photocatalytic degradation of phenol and benzoic acid using zinc oxide powders prepared by the sol–gel process, Alexandria Engineering Journal 52, 517–523.

Haggblom, M. M (1992) Microbial breakdown of halogenated aromatic pesticidesand related compounds. FEMS Microbiology Rev. 103:29–72.

Hansen TL, Schmidt JE, Angelidaki I, Marca E, Jansen JlC, Mosbaek H (2004), Method for determination of methane potentials of solid organic waste Waste Management 24 : 393–400.

Hanzel, J., Thullner, M., Harms, H., Wick, L.Y (2012) Walking the tightrope of bioavailability: growth dynamics of PAH degraders on vapour-phase PAH. Microb. Biotechnology. 5, 79–86.

Happold, F.C. and A. Key (1932) The Bacterial Purification of Gasworks' Liquors. The Action of the Liquors on the Bacterial Flora of Sewage. J. Hygiene 32:573-580.

Harwood, C.S., M. Rivelli, and L.N. Ornston (1986) Aromatic Acids are Chemo attractants for *Pseudomonas putida*. J. Bacteriol. 160:622-628.

He, Z. and J. Wiegel. (1995) Purification and Characterization of Oxygen-Sensitive Reversible 4-Hydroxybenzoate Decarboxylase from Clostridium hydroxybenzoicum. Europian Journal of Biochemistry . 229:77-82.

Hen Friman , Alex Schechter , Yeshayahu Nitzan , Rivka Cahan (2013) Phenol degradation in bio-electrochemical cells International Biodeterioration & Biodegradation, 84 , 155-160.

Hill, GA. & Robinson, C.W. (1975). Substrate inhibition kinetics: phenol degradation by *Pseudomonas putida*. Biotechnology and Bioengineering., 17:1599-1615.

Howard P.H. (1989). Handbook of environmental fate and exposure data for organic chemicals. Chelsea, Michigan, Lewis Publishers. 1:468-476.

Hungate, R. E. (1950) The anerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. 14, 1–49.

Hungate, R. E. (1969) A roll-tube method for cultivation of strict anaerobes. Methods Microbiol. 3B, 117–132

Hunter, J.V (1971) Origin of Organics from Artificial Contamination In: Organic Compounds in the Aquatic Environment.pp. 51-94. (S.J. Faust and J.V. Hunter, Eds.).Marcel Dekker: New York.

Hutchinson, D.H. and Robinson, C.W. (1988) 'Kinetics of the simultaneous batch degradation of p-cresol and phenol by *Pseudomonas putida*', Appl. Microbiol. Biotechnol., Vol. 29, pp.599–604.

Ingerslev F. Anf Nyholm N. (2000), Shake – Flask Test for determination of biodegradation rates of C- labelled Chemical at low concentrations in surface water systems. Ecotoxicol. Environ. Safety. 45, 274-283.

Jianhua Wanga,, Xuanxuan Ma, Sujing Liub, Pengcheng Sunc, Ping Fanc, Chuanhai Xia (2012) Biodegradation of phenol and 4-chlorophenol by *Candida tropicalis* W1 Procedia Environmental Sciences 16 ,299 – 303.

Kanekar, P.P, Sarnaik SS, Kelkar AS (1999) Bioremediation of phenol by alkaliphilic bacteria isolated from alkaline lake of Lonar, India. J Appl Microbiol 85:128S-133S

Kargi, F., Uygur, A., Baskaya, H.S (2005) Para-chlorophenol inhibition on COD, nitrogen and phosphate removal from synthetic wastewater in a sequencing batch reactor. Bioresour. Technol. 96, 1696–1702.

Khaled, M. Khleifat (2006) Biodegradation of phenol by Ewingella americana: Effect of carbon starvation and some growth conditions. Process Biochemistry, 41, 2010-2016

Kilby, B.A. (1948) The Bacterial Oxidation of Phenol to β-Ketoadipic Acid. Proc. Biochem. Soc., Biochem. J.43:V-Vi.

Kim, J.H., Oh, K.K., Lee, S.T., Kim, S.W. and Hong, S.I (2002) Biodegradation of phenol and chlorophenol with defined mixed culture in shake-flasks and a packed bed reactor. Process Biochemistry. 37: 1367-1373.

Kluge C, Tschech A, Fuchs G (1990) Anaerobic metabolism of resorcylic acids (m-dihydroxybenzoic acids) and resorcinol (1,3-benzenediol) in a fermenting and in a denitrifying bacterium. Arch Microbiol 155:68–74

Knoll,G. and J. Winter (1989) Degradation of Phenol Via Carboxylation to Benzoate by a Defined, Obligate Syntrophic Consortium of Anaerobic Bacteria. Appl. Microbiol. Biotechnol. 30:318-324.

Koch, B., Ostermann, M., Hoke, H. & Hempel, D.C. (1993). Sand and activated carbon as biofilm carriers for microbial degradation of phenols and nitrogen-containing aromatic compounds. Water Res., 25:1-8.

Konings,W.N., J.S. Lolkema, and B. Poolman (1995) The Generation of Metabolic Energy by Solute Transport.Arch. Microbiol. 164:235-242

Kroll R.G (1990) Alkalophiles. In: Edwards C (ed) Microbiology of extreme environments. Open University Press, Milton Keynes, UK, pp 55–92.

Kuever, I., 1. Kulmer, S. Jannsen, U. Fischer, and K.-H.Blotevogel (1993) Isolation and Characterization of a New Spore-Forming Sulfate-Reducing Bacterium Growing by Complete Oxidation of CatechoL Arch. Microbiol.159:282-288.

Kumaran, P., (1993) Specialised microbes in phenolic waste management. J. Appl. Microbiol. 20: 15-25.

Lack, A. and G. Fuchs (1992) Carboxylation of Phenylphosphate by Phenol Carboxy lase, an Enzyme System of Anaerobic Phenol Metabolism. J. Bacteriol. 174:36293636.

Laine M. And Jorgensen K. (1996). Straw compost and bioremediated soil as inoculate for the bioremediation of Chlorophenol – contaminated soil. Appl. Environ. Microbiol. 44, 1507-1513.

Lei, H., Ren, S., Wang, L., Bu, Q., Julson, J., Holladay, J., Roger, R (2011) The effect of reaction temperature and time and power input on microwave pyrolysis of distillers dried grain with solubles. Bioresour. Technol. / j.biortech.

Leonard, D. and Lindely, N.D (1998) Growth of Ralstoni eutropha on inhibitory concentrations of phenol- diminished growth can be attributed to hydrophobic perturbation of phenol hydroxylase activity. Enzyme Microbiology Technology.25: 271-277.

Levén Lotta, Anna Schnürer (2010) Molecular characterisation of two anaerobic phenol-degrading enrichment cultures International Biodeterioration & Biodegradation 64, 427-433.

Li, T., I.-G. Bisaillon, F. Lepine, R. Villemur, and R. Beaudet (1997) Partial Characterization of a Phenol Carboxylase Activity from a New Strain of Clostridium. In:Abstracts of the 97th General Meeting of the American Society for Microbiology 1997, Abstract Q-224,P. 492. American Society for Microbiology, Washington,D.C.

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.

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.

Lotta Leve´n_,Anna Schnu¨ rer (2005) Effects of temperature on biological degradation of phenols, benzoates and phthalates under methanogenic conditions. International Biodeterioration & Biodegradation 55, 153–160.

Lotta Levén , Karin Nyberg , Anna Schnürer (2012) Conversion of phenols during anaerobic digestion of organic solid waste- A review of important microorganisms and impact of temperature. Journal of Environmental Management 95, S99- S103.

Lovley, D.R. andD.I. Lonergan (1990) Anaerobic Oxidation of Toluene, Phenol, and p-Cresol by the Dissimilatory Iron-Reducing Organism, GS -15. Appl. Environ.Microbiol. 56: 1858-1864.

LovleyDR (2010) Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environ Microbiol Rep doi: 10.1111/j.1758-2229.2010.00211

Lu Yang, Yujing Wang, Jing Song, Wei Zhao, Xiaoli He, Jun Chen, Ming Xiao., (2011). Promotion of plant growth and in situ degradation of phenol by an engineered *.Pseudomonas fluorescens* strain in different contaminated environments. Soil Biology & Biochemistry 43, 915-922.

Lukyanova LD, Storozheva ZI, Proshin AT (2007). Corrective effect offlavonoid containing preparation extralife on the development of Parkinson's syndrome. Bull. Exp. Biol. Med., 144: 42-45.

Luoa Haiping, Guangli Liua, Renduo Zhanga, Song Jin (2009) Phenol degradation in microbial fuel cells, Chemical Engineering Journal 147: 259–264.

Maher, K.D., Bressler, D.C (2007) Pyrolysis of triglycerides for the production of renewable fuels and chemicals. Bioresour. Technol. 98, 2351–2368.

Margesin, R., Schinner F., (eds) (1999a) Cold-adapted organisms. Springer, Berlin Heidelberg New York.

Marrot, B. (2006). A. Barrios-Martinez, P. Moulin, N. Roche., Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor, Biochemical Engineering Journal 30, 174–183.

Master ER, Mohn WW (1998) Psychrotolerant bacteria isolated from Arctic soils that degrade polychlorinated biphenyls at low temperatures. Appl Environ Microbiol 64:4823–4829.

Maza-Márquez .P, Maria Victoria Martínez-Toledo , Jesús González-López , Belén Rodelas , Belén Juárez-Jiménez , Massimiliano Fenice (2013) Biodegradation of olive washing wastewater pollutants by highly efficient phenol-degrading strains selected from adapted bacterial community , International Biodeterioration & Biodegradation ,82, 192-198.

Md. Mahiudddin,. Fakhruddin A. N. M, and Abdullah-Al-Mahin , (2012) Degradation of Phenol via Meta Cleavage Pathway by Pseudomonas fluorescens PU1, ISRN Microbiology ,Volume 2012 Article ID 741820, 6 page,doi:10.5402/2012/741820

Meena Krishania , Virendra Kumar, Virendra Kumar Vijay, Anushree Malik (2013) Analysis of different techniques used for improvement of biomethanation process: A review. Fuel 106: 1–9.

Mitchell, P.1968. Chemiosmotic Coupling and Energy Transduction. Method for determination of methane potentials of solid organic waste. Waste Manage; 24,393–400.Glynn Research Ltd., Bodmin, England.

Mohn, W. W., and Tiedje, J. M.. (1992). Microbial reductive dehalogenation.Microbiol. Rev. 56:482–507.

Montserrat Tobajas, Victor, M., Monsalvo, Angel F. Mohedano, Juan J. Rodriguez (2012) Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by *Comamonas testosteroni*. Journal of Environmental Management 95, S116- S121.

Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167.

Müller R, Antranikian G, Maloney S, Sharp R (1998) Thermophilic degradation of environmental pollutants. In: Antranikian G (ed) Biotechnology of extremophiles. (Advances in Biochemical Engineering/Bio-technology, vol 61, Springer, Berlin Heidelberg, New York, pp 155–169.

Müller, R., Antranikian, G., Maloney, S., Sharp, R. (1998) Thermophilic degradation of environmental pollutants. In: Antranikian G (ed) Biotechnology of extremophiles. Advances in Biochemical Engineering/Bio-technology, Springer, Berlin Heidelberg New York, 61: 155–169.

Nair, C., Jayachandran, K and Shashidhar, S. (2008) Biodegradation of phenol, Afri. J. Biotechnol., 7 (25), 4951-4958,

National Research Council (1993) Insitu Bioremediation: When does it Work? ,National Academy Press, Washington DC.

Neujahr H.Y. and Gaal, A. (1973) Phenol hydroxylase from yeast. Purification and properties of the enzyme from Trichosporon cutaneum. Eur. Biochemistry. 35:386-400

Neujahr, H.Y. and A. Gaal. (1973) Phenol Hydroxylase fromYeast: Purification and Properties of the Enzyme from Trichosporon cutaneum. Europian Journal of Biochemistry . 35:386-400.

Neujahr, H.Y., S~ Lindsjo, and J.M. Varga (1974) Oxidation of Phenols by Cells and Cell-Free Enzymes from Candida tropicalis. Antonie Van Leeuwenhoek 40:209-216.

Niehaus F, Bertoldo C, Kähler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. Appl Microbiol Biotechnol 51:711–729 Chugunov VA, Ermolenko ZM, Martovetskaya II, Mironava RI, Zhirkova NA, Kholodenko VP, Urakov NN (2000) Development and

application of a liquid preparation with oil-oxidzing bacteria. Appl Biochem Microbiol 36:577–581

Niehaus F, Bertoldo C, Kähler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. Appl Microbiol Biotechnol 51:711–729.

Nilotpala Pradhan_, A.O. Ingle (2007) Mineralization of phenol by a *Serratia plymuthica* strain *GC* isolated from sludge sample. International Biodeterioration & Biodegradation 60, 103–108.

Norris PR, Johnson DB (1998) Acidophilic microorganisms. In: Horikoshi K, Grant WD (eds) Extremophiles: microbial life in extreme environments. Wiley-Liss, New York, pp 133–153.

Nuhoglu,A. And B. Yalcin (2005) Modelling of phenol removal in batch reactor process biochemistry.40(3-4): 1233-1239.

Ojumu, T.V., Bello, O.O., Sonibare, J.A. and Solomon, B.O. (2005) 'Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria', African Journal of Biotechnology, Vol. 4, No. 1, pp.31–35.

Oren A, Gurevich P, Azachi M, Hents Y (1992) Microbial degradation of pollutants at high salt concentrations. Biodegradation 3:387–398.

Paller, G., Hommel, R.K. and Kleber, H.P. (1995). Phenol degradation by Acinetobacter calcoaceticus NCIB 8250. J. Basic. Microbiol. 35:325- 335.

Pant.D,VanBogaertG,DielsL,Vanbroekhoven K (2010) A reviewof substrates use in microbial fuel cells(MFCs)for sustainable energy production. Bioresource Technol, 101:1533-1543. Excellent summary of the range of electron donors that can be oxidized in microbial fuel cells.

Parag R. Gogate (2008) Treatment of wastewater streams containing phenolic compounds using hybrid techniques based on cavitation: A review of the current status and the way forward .Ultrasonics Sonochemistry 15, 1–15

Patel, R.N., C.T. Hou, A.I. Laskin, and A. Felix (1982) Microbial Oxidation of Hydrocarbons: Properties of Soluble Methane Monooxygenase from a Facultative Methane-Utilizing Organism Methylobacterium sp. Strain CRL-26. Appl. Environ. Microbiol. 44:1130-1137.

Paula M. van Schie & Lily Y. Young (2000): Biodegradation of Phenol: Mechanisms and Applications,Bioremediation Journal, 4:1, 1-18

Peiro, A.M., Ayllon, J.A., Peral, J., Domenech, X (2001) TiO_2-photocatalysed degradation of phenol and ortho-substituted phenolic compounds. Applied Catalysis B: Environmental 30, 359-373.

Philipp, B., Schink B (1998) Evidence of two oxidative reaction steps initiating anaerobic degradation of resorcinol (1, 3-dihydroxybenzene) by the denitrifying bacterium Azoarcus anaerobius. J Bacteriol 180:3644–3649

Philipp, B., Schink B, (1998) Evidence of two oxidative reaction steps initiating anaerobic degradation of resorcinol (1,3-dihydroxybenzene) by the denitrifying bacterium Azoarcus anaerobius. J Bacteriol 180:3644–3649.

Prieur D, Marteinsson VT (1998) Prokaryotes living under elevated hydrostatic pressure. In: Antranikian G (ed) Biotechnology of extremophiles. (Advances in Biochemical Engineering/Biotechnology, vol 61) Springer, Berlin Heidelberg New York, pp. 23–35.

Qiu, Y.L., Hanada, S., Ohashi, A., Harada, H., Kamagata, Y., Sekiguchi, Y (2008) *Syntrophorhabdus aromaticivorans* gen. nov., sp nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. Applied and Environmental Microbiology 74, 2051-2058.

Quentmeier A, Friedrich CG (1994) Transfer and expression of degradative and antibiotic resistance plasmids in acidophilic bacteria. Appl Environ Microbiol 60:973–978.

Radwan, S.S., Al-Mailem D, El-Nemr I, Salamah S (2000) Enhanced remediation of hydrocarbon contaminated desert soil fertilized with organic carbons. Int Biodeterior Biodegrad 46:129–132.

Ramanand, K. and lM. Suflita (1991) Anaerobic Degradation of m-Cresol an Anoxic Aquifer Slurries: Carboxylation Reactions in a Sulfate-Reducing Bacterial Enrichment.Appl. Environ. Microbiol. 57:1689-1695

Roberts, D.J., Fedorak, P.M, and Hrudey, S.E. (1990) CO_2 Incorporation and 4-Hydroxy-2-Methylbenzoic Acid Formation During Anaerobic Metabolism of m-Cresol by a Methanogenic Consortium. Appl. Environ. Microbiol.56:472-478.

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.

Roya Pishgar, Ghasem Najafpour, Bahram Navayi Neya, Nafise Mousavi and Zeinab Bakhshi, (2011), Anaerobic Biodegradation of Phenol: Comparative Study of Free and Immobilized Growth, Iranica Journal of Energy & Environment 2 (4): 348-355.

Rozich A. F., and Colvin R. J. (1986) Effect of glucose on phenol biodegradation by heterogeneous populations. Biotech and Bioeng 28, 965-971.

Ryan, M. P., Pembroke, J. T., and Adley, C. C. (2007). Ralstonia pickettii in environmental biotechnology: Potential and applications. J. Appl. Microbiol.103, 754–764.

S. Chakraborty , T. Bhattacharya , T.N. Patel and K.K. Tiwari, (2010), Biodegradation of phenol by native microorganisms isolated from coke processing wastewater, Journal of Environmental Biology, **31** , 293-296

Saber Ahmed , Rasul M.G., R. Brown, M.A. Hashib,(2011). Influence of parameters on the heterogeneous photocatalytic degradation of pesticides and phenolic contaminants in wastewater: A short review. Journal of Environmental Management 92 ,311-330.

Saber Ahmed, M.G. Rasul , R. Brownb, M.A. Hashib.,(2011)Influence of parameters on the heterogeneous photocatalytic degradation of pesticides and phenolic contaminants in wastewater: A short review. Journal of Environmental Management 92 , 311-330.

Saghafi, S., Z. Bakhshi, G.D. Najafpour, E. Kariminezhad and H.A. Rad, (2010). Biodegradation of Toluene and Xylene in an UAPB Bioreactor with Fixed Film of *Pseudomonas putida*.

Sandhu A, Halverson LJ, Beattie GA (2009) Identification and genetic characterization of phenol-degrading bacteria from leaf microbial communities. Microb Ecol. 57(2):276-85

Santos, V.L., N.M. Heilbuth, D.T. Braga, A.S. Monteiro and V.R.J. Linardi: Phenol degradation by a Graphium sp. FIB4 isolated from industrial effluents. Basic Microbiol. 43, 238-248 (2003).

Schink B, Brune A, Schnell S (2000) Anaerobic Degradation of Aromatic Compounds. In: Winkelmann G (ed) Microbial degradation of natural compounds. VCH, Weinheim, pp 219–242.

Schink B, Pfennig N (1982) Fermentation of trihydroxybenzenes by Pelobacter acidigallici gen. nov. sp. nov., a new strictly anaerobic non-sporeforming bacterium. Arch Microbiol 133:195–201.

Schink Y, B. Philipp, J. Müller (2000) Anaerobic Degradation of Phenolic Compounds, Naturwissenschaften 87, 12–23, Springer-Verlag.

Schlegel HG (1992) Allgemeine Mikrobiologie. Thieme, Stuttgart

Schnell S, Schink B (1991) Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl CoA in Desulfobacterium anilini.Arch Microbiol 155:183–190

Schüler KH (1997) Isolierung und mechanistische Untersuchung der Resorcin-Reduktase in Clostridium KN245. Thesis, University of Constance.

Shashi Kumar, Deepika Arya, Abhinav Malhotra, Surendra Kumar , Brajesh Kumar (2013) Biodegradation of dual phenolic substrates in simulated wastewater by *Gliomastix indicus MTCC 3869*, Journal of Environmental Chemical Engineering 1 865–874.

Sheridan W. G., W. J. Jones, R. S. Wolfe, and M. T. Suidan (1985) Fundamentals Associated with Biodegradation of Phenols and Polycyclic N-armaric Compounds, Report to USEPA, Cooperative Agreement CR806819.

Shifu, C., Gengyu, C (2005) Photocatalytic degradation of pesticides using floating photocatalyst TiO_2 and SiO_2 beads by sunlight. Solar Energy 79, 1-9.

Shingler, V., Franklin, F.C.H., Tsuda, M., Holroyd, D. and Bagdasarian, M. (1989) 'Molecular analysis of a plasmid-encoded phenol hydroxylase from pseudomonas CF 600', J. Gen. Microbiol., Vol. 135, pp.1083–1092.

Siron R, Pelletier E, Brochu C (1995) Environmental factors influencing the biodegradation of petroleum hydrocarbons in cold seawater. Arch Environ Contam Toxicol 28:406–416.

Sowers, K. R., and Noll, K. M. (1995). Techniques for anaerobic growth, methanogenic archeae. In "Archaea, a Laboratory Manual," (F. T. Robb, A. R. Place, K. R. Soweres, H. J. Schreier, S. DarSarma, and E. M. Fleischmann, eds.), pp. 17–47. Cold Spring Harbor Laboratory Press, NY.

Sridevi, V., Chandana Lakshmi M.V.V., Manasa, M., Sravani, M., (2012) Metabolic pathways for the biodegradation of phenol, , international journal of engineering science & advanced technology, Volume-2, Issue-3, 695 – 705 , ISSN: 2250–3676.

Stapleton, R.D., Savage, D.C., Sayler, G.S., Stacey, G (1998) Biodegradation of aromatic hydrocarbons in an extremely acidic environment. Appl Environ Microbiol 64:4180–4184.

Stetter KO (1998) Hyperthermophiles: isolation, classification, and properties. In: Horikoshi K, Grant WD (eds) Extremophiles: microbial life in extreme environments. Wiley-Liss, New York, pp 1–24.

Sullivan, M. O., (1998) The degradation of phenol and mono-chlorophenols by a mixed microbial population. Ph.D.Thesis, Dublin City University. Ireland.

Taghreed Al-Khalid & Muftah H. El-Naas (2012): Aerobic Biodegradation of Phenols: A Comprehensive Review, Critical Reviews in Environmental Science and Technology, 42:16,1631-1690

Talley, J.W. and Sleeper, P.M. (1997) 'Roadblock to the implementation of biotreatment strategies', in Bajpai, R.K. and Zappi, M.E. (Eds.): Annuals of New York Academic of Sciences, Vol. 829, November 21, pp.17–29.

Tan, Y.; Wang, Z.; and Marshall, K.C. (1996) Modeling Substrate Inhibi tion of Microbial Growth. Biotechnol. Bioeng., 52, 602.

Tezcanli-Guyer, G., N.H. Ince (2004) Individual and combined effects of ultrasound, ozone and UV irradiation: a case study with textile dyes, Ultrasonics 42, 603–609.

Topp, E. and 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.

Tschech, A. and Fuchs, G. (1987) Anaerobic degradation of phenol by pure cultures of newly isolated Denitrifying Pseudomonads. Arch. Microbiol. 148:213-217.

Tschech, A., Schink, B. (1985) Fermentative degradation of resorcinol and resorcylic acids. Arch Microbiol 143:52–59

Tuan Nguyen Ngoc, Hsiao-Cheng Hsieh , Yi-Wen Lin , Shir-Ly Huang (2011) Analysis of bacterial degradation pathways for long-chain alkylphenols involving phenol hydroxylase, alkylphenol monooxygenase and catechol dioxygenase genes. Bioresource Technology 102 , 4232–4240.

Valenzuela, M.A., Bosch, P., Jiménez-Becerrill, J., Quiroz, O., Páez, A.I (2002) Preparation, characterization and photocatalytic activity of ZnO, Fe_2O_3 and $ZnFe_2O_4$. Journal of Photochemistry and Photobiology A: Chemistry 148,177-182.

Van Schie, P.M. and Young, L.Y. (1998) Isolation and Characterization of Phenol-Degrading Denitrifying Bacteria.Appl. Environ. Microbiol. 64:2432-2438.

Wang, K.W., Baltzis, B.C., Lewandowski, G.A (1996) Kinetics of phenol biodegradation in the presence of glucose. Biotechnology and Bioengineering 51, 87–94.

Wang, Y., Song, J., Zhao, W., He, X., Chen, J., Xiao, M., (2011). In situ degradation of phenol and promotion of plant growth in contaminated environments by a single *Pseudomonas aeruginosa* strain. J. Hazard. Matter. 192, 354–360.

Wang, Y., Tian, Y., Han, B., Zhaw, H. B., Bi, J. N., and Cai, B. L. (2007). Biodegradation of phenol by free and immobilized Acinetobacter sp. Strain PD12. J. Environ. Sci. 19, 222–225.

Weiner JM, Lovley DR (1998) Anaerobic benzene degradation in petroleum-contaminated aquifer sediments after inoculation with a benzene-degrading enrichment. Appl Environ Microbiol 64:775–778.

Whitehouse BG (1984). The effects of temperature and salinity on the aqueous solubility of polynuclear aromatic hydrocarbons. Mar Chem 14:319–332.

Whyte, L.G, Bourbonnière, L., Bellerose, C., Greer C.W. (1999) Bioremediation assessment of hydrocarbon-contaminated soils from the High Arctic. Bioremediation J 3:69–79.

Whyte, L.G., Hawari, J., Zhou, E., Bourbonnière, L., Inniss, W.E., Greer, C.W. (1998) Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic Rhodococcus sp. Appl Environ Microbiol 64:2578–2584.

Wick, L.Y., Shi, L., Harms, H (2007) Electro-bioremediation of hydrophobic organic soil-contaminants: a review of fundamental interactions. Electrochimica Acta 52, 3441-3448.

Wust, P.K., Horn, M.A., Drake, H.L., (2011). Clostridiaceae and Enterobacteriaceae as active fermenters in earthworm gut content. The ISME Journal 5, 92 - 106.

Y i-Tin Wang *et al*, Makram T. Suidan, John T. Pfeffer, and lssam Najam,(1989) The Effect of Concentration of Phenols on their Batch Methanogenesis, Biotechnology and Bioengineering, Vol. 33, Pp. 1353-1357 (1989),John Wiley & Sons, Inc.

Yi Li , Jing Li, Chao Wang, Peifang Wang (2010) Growth kinetics and phenol biodegradation of psychrotrophic *Pseudomonas putida* LY1. Bioresource Technology 101, 6740–6744.

Zhang T, Gannon, S.M., Nevin K,P., Franks, A,E., and Lovley, D.R.: (2010) Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environ Microbiol, 12:1011-1020.

Zhang, X. and I. Wiegel (1994) Reversible Conversion of 4-Hydroxybenzoate and Phenol by Clostridium hydroxybenzoicum. Appl. Environ. Microbiol. 60:4182-4185.

Zhao, C., Yang, Q., Chen, W., Teng, B. (2012) Removal of hexavalent chromium in tannery wastewater by Bacillus cereus. Canadian Journal of Microbiology 5: 23 - 28.

Zhou, J., Fries, M.R.,. Chee-Sanford, J.C., and. Tiedje, J.M. (1995) Phylogenetic analyses of a new group of denitrifiers capable of Anaerobic growth on Toluene and description of Azoarcus tolulyticus sp. Nov. Int.J. Syst. Bacterial. 45: 500-506.

Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan I,Stipek, S., Mikulikova, L., Popov P., (2001) Oxidative stress, metabolismof ethanol and alcohol related diseases. J. Biomed. Sci. 8, 59-70.