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Review Article

Biodegradation of Phenol: Mini Review

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Abstract This paper is a comprehensive review related to the biological degradation of phenol by microorganisms. The aromatic compound, phenol or hydroxybenzene, is produced industrially or naturally. Many microorganisms that are able to biodegrade phenol have been isolated and at the same time, the metabolic pathways responsible for these metabolic processes have been determined. A large number of bacteria were studied in detail especially, pure cultures as well as the pathways of aerobic phenol metabolism and the enzymes involved. Phenol oxygenation occurred as the initial steps through phenol hydroxylase enzymes leading to formation of catechol, pursued by the splitting of the adjacent ring or in between the two groups of catechol hydroxyls. Thus, the physical and chemical environments plus the chemical structures that affecting biodegradation processes are important determining factors for combating of pollution. This nature of chemical structure for the other aromatic compounds is also a main decisive factor of biodegradability.

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INTRODUCTION

Organic aromatic compounds are mostly pollutants involve a conceivable set of chemicals which can be very much risky to human health. Considerable of these are to. The utilization of carbon or energy sources by living cells is basic to all forms of life. As persist in the surroundings, they are able of long extent conveyance, biological in animal and human tissue and then being biomagnified in the food chain. The biodegradation of phenol and associated compounds has been a subject of scientific concern for many decades (Khleifat, 2010; Ahmad et al., 2017). Bacterial cells have over the centuries adapted to use the unprocessed organic chemicals located on earth man-produced chemicals, have resulted in environmental issues (Hill et al., 1996; Khleifat et al., 2007; Khleifat, 2007; Mishra and Kumar, 2017), because some organic compounds exhibit resistance or complete implacability to mineralization.

Severe exposure to phenol gives rise to disorders in the central nervous system, hepatic damage, anorexia, dermal rash, dysphasia, gastrointestinal disturbance, vomiting, weakness, weightlessness, and likely paralysis, cancer and genetofibre striation (Gonzalez et al., 2001). this results in the muscular spasms, break down and zombie. This results in a lowering in body temperature, known as a hypothermia. In addition, the mucosa appears very sensitive to. Phenol Impairments and trembles in muscle are also noticed. Severe exposure toexposure to phenol may cause weakness of myocardia. Skin burning features also occured by Phenol. Phenol including and erosion of the skin . Phenol exposure also leads to anaesthetic effect and may result in gangrene. Persistent subjection to phenol induces renal damage and salivation. Phenol is a leading pollutant comprised in the record of United Nations Environmental Protection Agency (EPA). It might be deadly by intake, inhalation and skin imbibition due its quick penetration to the skin (Khleifat, 2006; Abboud et al., 2007). It may give rise to acute irritation to the respiratory tract and eyes. It is listed as being ahuman carcinogenic and may be deadly to fish at low concentrations (5–25 mg/l). It has been observed that phenol inhibits photosynthesis of diatoms and blue-green algae at least at a concentration of 0.1 µg/ml. Phenol and its derivative catechol exhibit peroxidative capability, they are hepatotoxic and hematotoxic. It is a considered a mutagen and carcinogen to humans and different organisms (Gonzalez et al., 2001). It causes cause protein denaturation and general respons poisoning of protoplasm. Phenol might give rise to damage in peripheral nerve such as demyelination of axons.

Phenol its derivatives possess different levels of toxicity while their final fate in the environment is thus substantial (Bollag et al., 1988). In recent years, number of investigation has studied the

advanced processes in which phenolic contaminants are removed by using enzyme technologies (Ghioureliotis and Nicell, 1999). Phenol is used in surgery as an antiseptic agents and indicating that these compounds are also toxic to several microorganisms (EPA, 1979).

General Aspects

The source of phenol (Figure 1) in the environment is usually from natural materials,or man-made. It is the result of its processing and use in many applications such as wood burning, car exhaust, etc (Kumaran and Paruchuri, 1997; Khleifat, 2007). It was also found in air and water. Phenols are mostly found in water resulting from the discharge of industrial liquid waste. It was initially applied as creosote, in the raw state, to stop weathering railroad ties and shipwoods, and to reduce the smell of decomposition in sludge. Usually phenol is colorless at room temperature, and a white transparent powder or syrup liquid with when mixed water. The crystals turn pink into red in the air and are hygroscopic. Phenol contains a sweet tar like odour and is soluble in alcohol, petroleum and glycerol and to a lesser extent in water. Phenol is a component of coal tar as a natural source, coal tar results from the processing of coal and natural gas. It was detected among volatile components in watery manure at concentrations of 7-55 µg/Kg dry weight with rate of concentration in manure by 30µg/Kg dry weight (Kumaran and Paruchuri, 1997). The sources of human-made phenol include fossil fuel extracted industrial waste, pesticide manufacturing plants, the wood processing industry (Kumaran and Parachore, 1997), petroleum and petrochemical refineries, organic chemical manufacturing, pulp and paper factories, coal and tanning manufacturing, plastics and pharmaceutical industries (Lakshmi and Sridevi, 2015), as well as from agricultural runoff. Municipal sewage and chemical water leakage from many other industries liberate phenol and phenolic compounds into the environment (Lakshmi and Sridevi, 2015).

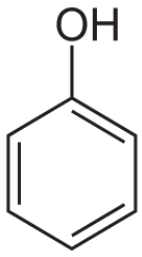

Figure1.Phenol chemical structure (source is https://en.wikipedia.org/wiki/Phenol).

As a result, to keep the soils and aqueous ecosystems safe, it has been imperative to treat industrial wastewater effluents for the sake of safe discarding the phenol to the surroundings (Khleifat et al., 2008a). Effective remediation processes for the elimination of phenol including ion exchange, activated carbon adsorption, chemical oxidation and liquid–liquid extraction; however, these chemical methods often suffer from need for large expenses. besides that the majority of these processes do not degrade phenol, but rather convert it to another stage, which leads to the formation of dangerous by-products (secondary pollution). In contrast, biodegradation is an alternative environmentally friendly and more cost-effective alternative. Thus, the biological treatment of phenol compound can be an increasingly important process in combating pollution (Wang et al., 2007; Khleifat et al., 2008b; Liu et al., 2009; El-Naas et al., 2009; El-Naas et al., 2010).

Mechanism of Phenol Degradation via Bacteria

Microbical cells evolved to devour the unprocessed natural chemicals, however, the considerable types of organic chemicals which are produced by human have caused environmental issues, due to impedance or complete resistance to mineralization by any microbial species (Khleifat, 2006). Phenol and its derivatives are widespread starting substances. They are secondary waste in the production of manufacturing and farming productions (Liu et al., 2009a). Phenol may be toxic to many aquatic species at levels as low as mg L^{-1} range and lead to taste and odour problems in drinking water at very low of concentrations (Chung et al., 2003; Zhao et al., 2009). Use of large quantities of Phenol in the United States and its potential toxicity made The US Environmental Protection Agency include it on Its list of priority pollutants (Agarwal and Ghoshal, 2008). The environmental cleaning-up of phenol via solvent extraction, adsorption, incineration, chemical oxidation, and biotic remediation process eprocesses bear serious drawbacks such as economic problems and the poduction of dangerous by-products (Zumriye and Gultac, 1999)). In general, bioremediation or biodegradation is preferred, due to low expenses and the complete biodegradation. There is much debate on whether to use indigenous or genetically modfied microorganisms (GEMs) in biodegradation. Government administrations are mostly disinclined to allow the release releasing of GEMs into the environment due to the potential of unpredicted unwanted ecological effects (Shourian et al., 2009; El-Naas et al., 2009). In several situations, the existance of substitutional, readily utilized carbon sources improved degradation, whilst in others the converse effect was perceived. Lactose at 1% concentration nearly fully inhibited the demolition of 212 mg L^{-1} phenol via a phenol-degrading bacterial consortium culture on a trickling filter (Hamdy et al., 1954). Likewisely, triphasic growth was reported when three substrates (glucose, lactose and acetate) were included together in a

mixed culture containing Pseudomonas sp. and Escherichia coli (Mateles and Chian,1969). The order of their usage was Glucose first, lactose second and acetate last. The relative numbers of Pseudomonas and coliforms varied in reaction to differing the concentrations of substrate.

The bactericidal action of phenol is grounded on its ability to break up cell membranes. Because the membrane is the sole partition between the exterior sphere and the cytoplasm, perturbation of membrane easily leads to cell death. Despite many of bacteria usually are bactericidal, There are many studies on bacteria that have shown mechanisms to survive and resist high concentrations of phenol. These mechanisms counteract the increase fluidity in membrane that results from the partitioning by phenol, including the isomerization of unsaturated fatty acids to trans-configuration, as presented by Pseudomonas putida during phenol degradation (Heipieper et aI., 1992). This organism rotates the 16-carbon length of cis-unsaturated fatty acids to the trans-configuration following exposure to phenol. Since the molecules of trans fatty acid chains can line up more closely together in a biological membrane than those in the cis configuration, therefore, the rigid membrane is formed for example, *P. putida* P8 cells adapted to using phenol as a carbon and energy sources can maintain its growth in the presence of 8 mM (750 mg/L) phenol concentrations, whereas P. putida P8 cells which not acclimitized to phenol were unable to grow on concentrations higher than 2.6 mM (250 mg/L) A different mechanism is to raise the ratio of saturated fatty acids to unsaturated acids in the membrane as reported for Escherichia coli K-12. This bacterium was able to grow on concentrations of phenol up to 1000mg/L (10.6 mM) by changing unsaturated fatty acids to saturated ones (Keweloh et aI., 1991). Like trans fatty acids, the chains of saturated fatty acids can align closer together in the membrane, which may prevent the increased membrane fluidity induced by phenol. Therefore, it is very important to isolate this kind of bacteria capable of thriving under high phenol concentrations and with other organic solvents, because such bacterial species would be beneficial in biodegradation processes where these compounds usually being toxic or inhibitory (Heipieper et al., 1994).

As long as the majority of the world's population (60-80%) depend on medications to treat diseases (Tarawneh et al., 2010; Zeidan et al., 2013; Majali et al., 2015; Alrawashdeh et al., 2019; Khleifat et al., 2019) . However, in addition to the environmental activity, which is the disposal of these organic pollutions by microbes, they are useful as models for identifying many of various active biological compounds of medicinal plants ((Tarawneh et al., 2011; Tarawneh et al., 2019). For example, phenolic compounds and essential oils play critical functions such as antimicrobial and antioxidant activities. Bacteria have extensive activities in different applications including environmental such as biosorption of metals (Khleifat and Abboud, 2003; Khleifat, 2006a-c; Khleifat et al., 2006a; Abboud et al., 2009; Khleifat et al., 2006a-d; Althunibat et al., 2016). The medical characteristics such as resistance to antimicrobial agents, production of some antibiotics as well as their capability of nanoparticles formation (Al-Asoufi et al., 2017; ALrawashdeh et al., 2019). Finally, the biotechnological uses such as production of enzymes and fermentation products (Khleifat et al. 2009; Aljundi and Khleifat, 2010; Allimoun et al., 2015; Al-Limoun et al., 2019).

The main method for removing phenols from the environment include its biotransformation by bacteria in water and soil (Krijgsheld and van der Gen, 1986). The discovery of bacterial degradation of aromatic compounds (Rogoff, 1961; McKinney et al., 1956), lead to the extensive studies of biodegradation using bacteria. A large number of bacteria were studied in detail in particular, purely aerobes cultured and pathways of aerobic phenol metabolism and the enzymes involved. Recent, research has conferred a great perception into the genes that express these phenol hydroxylase enzymes. Many of these enzymes, especially single flavopotein monooxygenases or multicomponent enzymes, which are similar to other oxygenases that are responsible for oxidation of aromatic compounds such as phenol, substituted phenol, benzene and toluene. More interesting topics about the roots and development of these enzymes is expected as more gene sequences and thus more types of oxygenase enzymes being available (Khleifat et al., 2019). The mainstream emphasis on investigation using pure bacterial cultures, with fewer studies dealing with plant growth promoting bacteria (Orhan, 2016).

The phenol-adapted bacteria were found to be able to use a range of substrates, involving hexose and pentose sugars, substitutive benzoic acid compounds, minimally 13 amino acids, simple aliphatic compounds, cresols, benzyl alcohol and numerous dimethyl phenols (McKinney et al., 1956, Khleifat, 2006a). Many bacteria are effective in degrading phenol involve various species of Burkholderia, Rhodococcus, Ewingella, Pseudomonas, Vibrio, Alcaligenes, Azotobacter, Spirillum, Xanthomonas, Flavobacterium, Acinetobacter, Chromobacter, Bacillus, and Corynebacterium. Members of the actinomycete genus, Nocardia, have also been found to degrade phenol (Kobayashi and Rittman, 1982; Knackmuss

and Hellwig, 1978). In terms of the ability to employ a wide variety of substrates, *Pseudomonas* species were the mostly reported early as versatile of degraders of phenol. It was reported that an isolate of unidentified gram-changeable bacillus obtained from pentachlorophenol was able to degrade different multi-halogenated phenols; it was incapable of breaking down phenol, and to a limited extent, it degraded only monochlorophenols (Chu and Kirsch, 1973; Khleifat et al., 2015). Most of the studies pertaining to bacterial metabolism of phenol and related compounds were aerobically performed. It was reported that such biodegradation of these aromatic compounds requires either the existance of oxygen or an oxygen-harbouring substance on the substrate (Bouwer and McCarty, 1983). This, hypothesis was founded on what was well-known pertaining to the ring cleavage mechanism of the aromatic compounds, which mostly needs the existence of two atoms of oxygen on the aromatic ring ortho to each other.

During phenol biodegradation, the first step of the aerobic pathway, a molecular oxygen is used by the phenol hydroxylase enzyme to add a second hydroxyl group in the ortho-position to the group previously present. This step requires reduced pyridine nucleotides ($NADH_2$). This generates, two different pathways, relying on the organism being used, can then break up L- dihydroxybenzene (catechol molecule). In general, aromatic ring cleavage cannot occur without the presence of oxygen molecules (Khleifat, 2007a ; Al-Khalid and El-Naas, 2012). This method, cleavage of rings through oxygenation, seems to be an ability of each aerobic microorganism (Khleifat, 2006a). During biodegradation of aromatic compounds they are transformed to dihydroxy derivatives of either ortho or para before ring cleavage. The process of ortho hydroxylation of phenols causes production of analogical catechols. A purified hydroxylase enzyme for phenol was obtained from the bacterium Brevibacterium fuscum, isolated from soil (Nakagawa and Takeda, 1962). This enzyme can hydroxylate orcinol, the three cresols, 2-amino-m-cresol, all three aminophenols, P-napthol and phloroglucinol. Thus, the extensive substrate specificity of phenol hydroxylase enzyme is not exceptional property. The enzyme needs two atoms of oxygen and a cupric ion per each phenol to be hydroxylated (Al-Khalid and El-Naas, 2012). In general, hydroxylase enzymes are inducible. After the formation of the corresponding catechol during phenol degradation a cis,cis-muconatering is formed beyond the ring cleavage (Khleifat, 2006a). This intermediary compound was generated during phenol metabolism in many soil microorganisms including bacteria, yeasts and mycelial fungi (Varga and Nejuahr, 1970). Various microorganisms, are incapable of degrading catechol or its frequent predecessors (Cook and Cain, 1974). In these conditions, biodegradation of phenol proceeds using protocatechuate, rather than via catechol. Bacteria and fungi generally use the protocatechuate pathway (Cain et al., 1968). The next step after the creation of cis,cis-muconic acid in the main catalyzing pathway for phenol is the formation of ketoadipate, mostly by a muconolactone (Khleifat, 2006a). The step of lactonization that includes protocatechuate differs among different microorganisms including bacteria and fungi (Chambers and Kabler, 1964; Dagley, 1971). The 'ortho' cleavage lactonization is used by bacteria to shape a ß -carboxymuconolactone, whilst yeast generate the γ-isomer. The last step in the metabolism of phenol includes the cleavage of ß-ketoadipate followed by formation of succinate and acetate.

The nature of chemical structure of the aromatic compounds is a main factor determining the biodegradability. Rates and degrees of biodegradation of aromatic-substrate have been stidied with various substituents on the benzene ring, different substitutents or multiples of same, complexity and size of substituents (Tabak et al., 1964). For example, the extent of degradability of phenol using bacteria was lowered by the appearance of diphenyls, saturated rings, increasing number of substituents and phenolic compounds with chloro-, nitro-, or amino- groups attached to rings (Van Schie and Young, 2000). The nature, concentration and availability of inorganic nutrients may also affect degradation amounts of xenobiotic compounds by microorganisms, as shown with bioactivity growth rates (Liu et al., 2009b). However, little detailed information is specific to biodegradation of phenol and chlorinated phenols is present in the literature. Oxygen is one of the essential nutrient when a chemical reaction in a dissimilative metabolic pathway is restricted to aerobic conditions. Nitrogen is a general restricting factor for bacterial growth in natural habitat (waters and soil). It was not unexpected, then, that reduction of the nitrogen stock in eutrophic-lake-water samples reduced the regular rate of phenol degradation. It was also noticed that rates of biodegradation boosted when eutrophic lake water was supplied with inorganic nutrient solutions including some inorganic salts (Rubin and Alexander,1983). Each of these growth aspects must be optimized for the chosen organism to obtain the best degradation rate for the selected organic compounds. Improving the concentration of substrate during biodegradation of phenol is substantially crucial because phenol biodegradation via bacterial cells was usually known to be inhibited by phenol itself, particularly, with high concentrations. This also applies to those species that potentially can be substrate users. Adjei and

Ohta, (2000) reported that phenol was a cyanide utilization inhibitor of the Burkholderia cepacia strain C-3. A kinetic study of P. putida MTCC 1194 , using 1000 and 500 mg/l as initial concentrations of phenol and catechol, respectively. showed an extended lag phase because of the high phenol concentration (Li et al., 2010). As the initial concentration raises, the delay in initiation of growth was longer probably because of the inhibitory effect of the phenol. The growth of bacteria cannot be detected at concentrations 1200 mg/l of phenol even following 20 days of incubation. For Catechol, bacterial strain growth was inhibited upon exposure to 600 mg/l. According to Wang et al., (2007), little information is available on bacteria and their resistance to high concentrations of phenol as well as metabolic activity. Therefore, there is still a need to isolate this phenol-degrading bacteria that can grow and thrive on high concentrations of phenol. In this regard, two novel phenol-degrading bacteria were reported, Acinetobacter sp. strain PD12 and *Ewingella americana* isolated from different activated sludge locations (Khleifat, 2006a; Bi et al., 2007; Li et al., 2011)). Phenol toxicity prevents or slows metabolic reactions depending on the exposed microorganisms and the concentrations of specific toxicants. Toxicity is induced by an obstruction that may result from overall physical perturbation of the bacterial cell structure or hindering its enzymatic activity (Agarry et al., 2008). The bacterial concentration is another agent could be a determining factor for the gross ability of biodegradation.

Plant growth promoters capable of degrading phenol(s) have also been isolated and identified. Members of these isolated bacteria have been reported to use phenols, though slowly (Zhuang et al., 2007; Yamaga et al., 2010). However, there are little data available concerning biodegradation of phenols by plant growth promoters isolated from soil.

Biodegradation of Phenol by Plant Growth Promoting Bacteria

There are few data available pertaining to biodegradation of phenols by plant growth promoters. The disappearance of phenol by isolated plant growth promoters was not correlated with the occurrence of phenol in the field (Ehrlich et al., 1982; Wang et al., 2011; Imran et al., 2014; Pradeep et al., 2015; Silambarasan and Vangnai, 2016; Numan et al., 2018). In the case of rhizoremediation the rhizosphere bacteria in the atmosphere can steadily decompose organic pollutants during by using of nutrients from plant roots and exchanging oxygen in the rhizosphere (Chaudhry et al., 2005). It was reported that, introduction of the plant growth promoting bacterium, *Pseudomonas fluorescens* strain P13, (transconjugant P13 strain) into a soil that was artificially spiked with phenol led to the growth promotion of the corn and in situ biodegradation of phenol concomitant with the increase in plant biomass and reduction of soil content of phenol (Yang et al., 2011). *Ochrobactrum anthropi* as plant growth-promoting bacterium was able to degrade 94 % of 100 mg/L phenol in 12 days (Imran et al., 2014). The presence of *Ochrobactrum anthropi* in rhizosphere caused significant increase in plant tallness, biomass and the uptake of nitrogen as comparing to inoculates free plants. These results, indicate the possibility of exploiting the potentials of the two to support plant growth and biological decomposition of phenol by these bacteria.

These researchers were able to isolate 41 species of bacteria from soil sampled from Al-Ghwair site, Karak, Jordan. Out of 41 only 16 bacterial species were able to have at least one plant growth promoting characteristic and most of them were aerobically able to use phenol as a sole carbon and energy sources. (Ehrlich et al., 1982; Godsy et al., 1983; Khleifat, 2007b; Al-Khalid and El-Naas, 2012). There is large effort to identify microorganism, which are capable of flourishing on elevated concentrations of pollutant organic compounds particularly, aromatic ones (Nair et al., 2008; El-Naas et al., 2009; Kumar and Kumar, 2005), like phenol. In general, mineralization is surpassed, due to minimal in costs and achievement of full biodegradation. There is frequent debate over whether to employ innate or genetically modified bacteria (GMB) in the biodegradation of organic pollutants such phenol (Al-Khalid and El-Naas, 2012). Government agencies are disinclined to liberate GMB into the environment, due to the potency of unpredicted negative environmental effects (Lob and Tar, 2000). The use phenol or other aromatic compounds as the single carbon and energy source by Ewingella americana has not been stressed . The manner of catechol degradation, availability of nutrient (carbon and nitrogen sources), the accessibility of toxins and optimal physical parameters (i.e., pH, temperature and shaking rate) could have an effect on the bacterial growth using phenol as growth substrate (Khleifat et al., 2006a). It appears that biodegradation of phenol may occur at room temperature, The optimum temperature for *E. americana* cell growth was 37 ^{o}C.

Therefore, temperature has an effect on the biodegradation process of aromatic compounds, It is believed that instantaneous subjection to temperatures above 35°C could be detrimental for bacterial enzymes responsible for the cleavage of benzene ring; this enzyme is known to be a key

step during the biodegradation of such compounds (El-Naas et al., 2009). This means that the appropriate temperature leads to the best conditions for phenol degradation or this result may only be due to the influence of temperature on general enzyme activities (Kılıc, 2009). Previous reports have mentioned that the temperature factor may play a more important role than nutritional factors in the facilitating of the degradation of Phenol (Bhatt et al., 2007). In general, each bacterium has a specific incubation temperature range for its growth. Furthermore, exposing of bacteria to temperatures lower than 30°C will lead to slowing down the bacterial activity and thus increasing the inhibition effect of phenol degradability by the bacteria, particularly, for high concentrations of phenol. The majority of the biodegradation studies on phenol had been conducted in the temperature ranged between 25–35°C (Khleifat et al., 2010). It is known that phenol degradation enzymes are usually have their optimal activities at a specific pH, depending on the bacterial species. For instance, the pH ranges between 8 and 11 for phenol biodegradability by *Halomonas campisalis* (Khleifat, 2006a; Lucas et al., 2008; Liu et al., 2016) and the optimal pH for phenol degradation by *E. americana* and *Klebsiella oxytoca* was 7.5 and 6.8, respectively (Khalifat et al., Unpublished data). Although there is large amount of research regarding the biological treatment of phenols, there is a lack of information pertaining the biodegradation of phenol by Plant growth promoting bacteria (PGPB). Due to the oxygen requirement at early step in this pathway, biodegradation of phenol through aerobic and anaerobic conditions could be occurring. However, its degradation is favored at aerobic rather than anaerobic conditions. Investigation of seawater sludge have shown that a bacterial consortium degrades phenol with better than do isolated single bacterial strains (HuiJie et al., 2011). Therefore, a mixture of bacterial strains is usually preferred to implement the degradation of such compounds aerobically. Single bacterial isolates usually have unlimited ability for complete degradation of the phenol as well as it's substitutions. There is great importance in isolating bacteria with the ability to thrive with high concentrations of phenol compounds studied here (Hill et al., 1996; Lob and Tar, 2000 Bastos et al., 2000).

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