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Microbial Degradation of Benzene : A Review

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ABSTRACT: Benzene is a recalcitrant compound and is classified as priority pollutants. Releasing benzene into the environment whether accidentally or due to human activities is a main cause of soil pollution. Cleaning up these pollutants from the environment is a vital world problem. Bioremediation has become a major method employed in refurbishment of benzene polluted environments that makes use of natural microbial biodegradation activity. benzene utilizing microorganisms are ubiquitously distributed in petroleum contaminated soil. They naturally biodegrade pollutants and thereby put an end to them from the environment. Removal of benzene pollutants from the environment by applying oleophilic microorganisms is eco friendly and economic. This microbiological decontamination is asserted to be an efficient and versatile alternative to physicochemical treatment. This article provides an overview about bioremediation for benzene pollutants with the help of microbial isolates that are isolated from the petroleum contaminated soil.

KEYWORDS: Benzene, Biodegradation, petroleum contaminated soil, Petroleum degradation, pollution,.

I. INTRODUCTION

Petroleum-based products are the primary energy source for industry and everyday life. Accidental spills and leaks are common during the exploration, production, and refining processes. Petroleum and petroleum products are transported and stored. The quantity of natural crude oil seepage was believed to be in the billions of dollars. with a range of uncertainty of 600,000 metric tonnes per year and a production capacity of 200,000 metric tonnes peryear [1]. The unintentional or intentional release of hydrocarbons into the environment is a major source of water and soil contamination [2]. Because pollutants accumulate in animals, soil pollution with hydrocarbons causes considerable damage to the local ecology [3]. Contamination by petroleum hydrocarbons is a big concern all around the world. The use of these toxic substances in transportation and electricity generation continues unabated. continues to be the primary source of pollution [4] Benzene is a raw chemical that is utilized as a solvent or chemical intermediate, as well as a key component of fuels. Benzene is an industrialized chemical that is used to make plastics and certain types of rubbers, as well as detergents, medicines, and pesticides. It can detect crude oil, gasoline, and cigarette smoke, among other things. Benzene is a contaminant that can be found in the air, soil, and water Due to its extensive use, benzene is a widespread anthropogenic contaminant in aqueous environments [5]. In lymphocytes of patients who have been exposed to benzene for a long time, chromosomal abnormalities, sister chromatid exchange, and micronuclei development have been observed [6]. Benzene is a pervasive pollutant found in a variety of businesses and occupations, including oil and gas production and refining, distribution, sale, manufacturing and use of petroleum products, coke production, the manufacture and use of chemical products, automobile repair, shoe production. In these scenarios, the exposure will not usually be to pure benzene. Because it is liquid, poisonous, carcinogenic, and one of the most stable aromatic compounds in ground and surface water, benzene is a major source of concern. Microbial degradation of benzene in an aerobic environment has been successful, however poorly biodegraded in anaerobic conditions [7]. Although some bacteria have been reported to degrade petroleum components completely within a few days under culture condition but not as effective as expected in practical needs [8; 9]. There is potential for a study on removing benzene using a more dependable technology, such as bioremediation, which has already been demonstrated to be cost-effective and environmentally acceptable for removing toxins from contaminated environments [10; 11]. Bioremediation is the process of manipulating environmental factors to facilitate microbial growth and increased pollution breakdown while causing no harm to the environment [12]. The only major limitation of bioremediation is the slow rate of degradation, so much research is being done to improve the rate of degradation through the selection of suitable microorganisms, process optimization, and the selection of good reactor systems with suitable packing materials such as agro waste, wood charcoal, PUF, and other [10; 11; 13; 14; 15]. Hence, to effectively implement and

improve the overall remediation process, we need to isolate more microbes with novel enzyme activities that enhance the microbial benzene breakdown.

II. ESTIMATION OF BENZENE DEGRADATION

In the fields of bioremediation, wastewater treatment, and fermentation processes, microbial growth on substrate mixtures is a hot topic of research. However, thorough mathematical modeling of the biodegradation of numerous substrates has been extremely difficult. Aerobic benzene-degrading bacteria are common and have been recognised for a long time; in fact, the first report on aerobic benzene-degrading microorganisms was published about 100 years ago [16]. The review by Weelink and coworkers on benzene degradation by chlorate reduction is excellent and comprehensive (2010). Bielefeldt and Stensel (1999) were the first to quantify the biodegradation of a combination of five chemicals. Deeb and Alvarez-Cohen (1999) concentrated their efforts on studies on determining the effect and interplay of the variables On the biodegradation of benzene, toluene, and ethylbenzene Isomers of xylene hydrocarbons.

The nitration process can be used to evaluate benzene and toluene. The vapors are absorbed in nitric-sulfuric acid, and the dinitro compound is colorimetrically determined after reaction with methyl ethyl ketone [17]. In the presence of its homologs, particularly toluene and xylene, the standard chemical approach for determining benzene is ineffective. When benzene is mixed with unknown concentrations of other chemicals, such as its homologs, physical methods for detecting benzene, such as fractionation or ultraviolet absorption [17], cannot be utilized to estimate the benzene concentration. determining the presence and concentration of benzene in combinations of various materials, which is a regular occurrence in industry. The process entails nitration, ether extraction, color development with butanone and alkali in an alcoholic solution, removal of color due to interfering chemicals with acetic acid, and comparison of the residual color [17].

For estimation of benzene degradation, Das and Mukherjee found crude petroleum oil from petroleum-contaminated soil in North East India [18]. *Acinetobacter* sp. was discovered to be capable of using n-alkanes with chain lengths of C10– C40 as a sole carbon source [19]. *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia*, and *Mycobacterium*, which were isolated from petroleum-contaminated soil, were found to be potential hydrocarbon degraders [20]. The organism of bacillaceae species *Bacillus flexus* degraded 76.757% of benzene from a starting concentration of 10 M over a 7-day period, according to Mukherjee S. *et al.*, (2019). According to Ahmed A *et al.*, (2014) *E. cloacae* E1 is capable of degrading benzene derivatives and other aromatic compounds in 30 days.

The 2, 6-Dichlorophenol Indophenol (2,6-DCPIP) oxidation test was used to confirm the presence of hydrocarbon breakdown For this test, an electron acceptor dye. According to Bidoia [21], the degrading capacity of bacterial cultures is demonstrated by the total reduction of DCPIP in 75 hours. 87 hours for old oil, 125 hours for semi-synthetic oil For synthetic oil, the time is 138 hours. Bacteria from the genus *Burkholderia*, *Pseudomonas*, and *Serratia* are bacteria that can cause infections. breakdown a variety of hydrocarbons with a high molecular weight Dual Purpose Kerosene, Premium Motor Spirit (PMS) (DPK) and LPFO (Low Pour Point Fuel Oil) [22]. There are numerous species of *Bacillus*, *Burkholderia*, *Micrococcus*, *Proteus*, *Pseudomonas*. Several authors have reported on the use of hydrocarbons. via DCPIP oxidation [23; 24; 25].

III. FACTORS AFFECTING BENZENE DEGRADATION

Many limiting factors that affect the biodegradation of petroleum hydrocarbons have been identified, many of which have been described by Brusseau [26]. The petroleum composition and inherent biodegradability The first and most serious contaminant is hydrocarbons. When determining the suitability of a remedial strategy is to be evaluated Among physical factors, temperature plays a major role in hydrocarbon biodegradation directly impacting pollutant chemistry as well as influencing the microbial flora's physiology and diversity Atlas [27] discovered that the viscosity of the liquid decreases at low temperatures. While the volatility of the toxic molecular weight oil increased, the variability of the toxic low molecular weight toxic low molecular weight oil decreased. The amount of weight of hydrocarbons was lowered, which delayed the development of biodegradation. The concentration of hydrocarbons is also affected by temperature [28].

IV. MECHANISMS OF ANAEROBIC BENZENE ACTIVATION

Since the end of the 1980s, many people have tried to understand the activation process. Generally, the benzene molecule is thermodynamically strong because of the symmetric p-electron system of the benzene ring, as well as the absence of potentially unstable or reactive elements substituents. Many compounds which are alkylated aromatic

hydrocarbons, e.g. toluene and xylenes, are activated under anoxic conditions by a reaction in which fumarate is added to the alkyl side-chain of the aromatic ring [29]. Rather stable benzene radical is thought to be produced in the first phase of this reaction. Benzene may be triggered similarly. The production of benzene radical as a reactive intermediate, on the other hand, For energy considerations, an intermediate for later methylation and other reaction is unlikely. The extraction of hydrogen from benzene would necessitate the use of a catalyst. higher than 460 kJ mol⁻¹ activation energy, which is Approximately 100 kJ mol⁻¹ greater than the creation of Alkylated benzene derivatives yield a benzyl radical [30; 31]. The significance of this for the environment requires elucidating the anaerobic benzene degradation processes. The procedure and any potential biochemical unique characteristics [32]

here, we mention three mechanisms that received a lot of attention,

1. anaerobic hydroxylation of benzene
2. Friedel-Crafts-type methylation of benzene
3. carboxylation of benzene

1. Benzene hydroxylation

Because the redox potential of benzene hydroxylation by hydroxides derived from water is relatively low, it provides an appealing model for benzene activation [31]. As a result, even under sulfate-reducing or methanogenic circumstances, it's possible. Phenol is mineralized from both facultative and completely anaerobic bacteria, and the breakdown mechanism involves a carbon dioxide-dependent carboxylation of the aromatic ring to 4-hydroxybenzoate, followed by transformation to benzoyl CoA [33; 34; 35; 36; 37; 38]. In one of the first publications on anaerobic benzene degradation, hydroxylation of benzene was postulated as an early reaction mechanism for benzene activation [39]. The oxidation of iron in the sample during work-up presumably produced hydroxyl radicals, which subsequently interacted quickly with benzene. Before taking a sample, make modest amounts of phenol. In this process analysis The production of 2-hydroxybenzoate and 4-hydroxybenzoate The formation of hydroxybenzoate from benzoate was also explained [40]. These findings suggest that distinguishing both biotic and abiotic phenol production in very acidic environments is difficult. lowered the number of culture samples, most likely avoiding any obvious results proof for benzene hydroxylation in anoxic conditions metabolite analysis was used to determine the circumstances. As a result, methodological techniques are required. Substrate consumption assays using two sulfate-reducing benzene-degrading cultures indicated that phenol is either not used at all [40] or is only ingested in little amounts. Musat and Widdel (2008) found that after a specific lag phase, the product is consumed [31]. suggesting that phenol is probably a carcinogen. an intermediate in the breakdown of benzene. Zhang and colleagues have mentioned phenol is a valuable metabolite during the growth of an isolated *Geobacter* conducting a sequence of specified strain on benzene under iron-reducing situations [32]. Gene upregulation lends credence to this method. for phenol metabolism throughout the breakdown of benzene and its subsequent inhibition once they have been deleted genes, as previously stated. Furthermore, these authors has been shown that water is the source of oxygen for the group of hydroxyls. According to these findings, hydroxylation possibly the first stage of benzene decomposition, The main actors are thought to be the *Geobacter* species [32; 41].

2. Benzene methylation

When using the unique biological methyl donors S-adenosyl-methionine or methyl-tetrahydrofolate, benzene methylation with the Friedel-Crafts-type process is exergonic [42]. This opens the way for a new anaerobic benzene activation hypothesis. Bone has been found to have S-adenosyl-methionine-dependent alkylation of benzene (and substituted aromatics) in bone marrow [43]. The anaerobic reactivity of the non substituted aromatics naphthalene has also been proposed [44]. If anaerobes methylate benzene, the reaction product toluene can be further activated by adding fumarate to the methyl group of toluene, which is mediated by one of the enzymes benzyl succinate synthase (BSS), resulting in the distinctive intermediate benzyl succinate. Benzyl succinate synthase had been around for a while. identified in many pure and anaerobic toluene-degradation anaerobic toluene-degradation anaerobic toluene-de fumarate addition appears to be beneficial in mixed cultures. Anaerobic toluene degradation has a one-of-a-kind activation mechanism [29]. PCR primers have also been created for the gene encoding the protein component benzyl succinate synthase A, which includes the reactive center [45]. The authors concluded that there are two degradation pathways: (1) a methylation pathway led directly to toluene with subsequent transformation to benzoate in the nitrate-reducing and methanogenic culture, and (2) a hydroxylation pathway leads to phenol with subsequent transformation to benzoate in the nitrate-reducing and methanogenic culture. Only in the methanogenic environment can benzoate be formed. culture. This notion was backed up by research. CSIA was utilized to characterize the first step of the process. activation of benzene in several cultures [46; 47; 48]. As a result, more research is required to demonstrate definitively that benzene may be methylated in nitrate-reducing circumstances. Toluene cannot be degraded by some highly

enriched purely anaerobic benzene degrading cultures [40; 49; 50] ; Benzene biomethylation is unlikely to result in the formation of The activation mechanism of toluene.

3. Benzene carboxylation

Benzene carboxylation, like hydroxylation and methylation, is somewhat similar to thermodynamic equilibrium, depending on the carboxyl donor, making it possible to use even in sulfate-reduced or sulfate-free environments cultures that produce methane [50]. In addition, for some non-substituted aromatic compounds, such as naphthalene or phenanthrene [50; 51], the carboxylation process for ring activation was proposed, implying that carboxylation could be an essential activation mechanism for rings. non-substituted aromatic compounds degradation in the world wild. In their highly enriched aquatic sulfate-reducing culture, Phelps and colleagues (2001) discovered deuterated benzoate (D5) as the sole intermediary of deuterated benzene (D6) degradation. Laban and colleagues (2010) used a combination of metagenomics and metaproteomics to analyze their highly enriched iron-reducing benzene-degrading culture. Benzene was used to grow the subcultures. As single substrates, phenol or benzoate, and peptide Following that, sequences were determined based on a metagenome that had already been sequenced Proteins comparable to the subunits of phenyl phosphate carboxylase *Azoarcus* sp. strain EbN1 PpcA and PpcD, as well as the *Geobacter* metallic reduced benzoate-CoA ligase was discovered. When anaerobic benzene breakdown occurs, this gene is selectively expressed. The authors proposed a solution based on their findings. that benzene is carboxylated directly by a putative carboxylase Benzene carboxylase in anaerobic conditions. The benzoate that has been created A benzoate-CoA ligase could further activate it. benzoyl-CoA. However, a test of enzyme activity for the ultimate, potential anaerobic benzene carboxylase has not yet been possible to prove this hypothesis.

List of percentage biodegradation of benzene by respected microorganisms.

Sr no.	Organisms	Degradation of hydrocarbons (benzene)	Time period	Reference
1	<i>Bacillus flexus</i>	76.757%	7 days	[6]
2	<i>bacillus</i> S35	85%	10 to 15 days	[52]
3	<i>bacillus</i> S6	71%	10 to 15 days	[52]
4	<i>enterobacter cloacae</i>	57.94%; 62.43%; 76%, 53%; 70%; 67.43%	1 hours and more; 15 to 20 days; 20 days; 3 days;	[53; 54; 55; 56]
5	<i>pseudomonas putida</i> F1	0.75%	more time required	[57]
6	<i>pseudomonas putida</i>	0.75%; 60%	5 to 7 days; 8 to 10 days	[58; 59]
7	<i>pseudomonas aeruginosa</i>	60%; 60%; 94%	8 to 10 days; 8 to 12 days; 50 to 80 days	[59; 60; 61]
8	<i>pseudomonas fluorescens</i>	0.83%; 40%	5 to 7 days; 8 to 10 days	[58; 59]
9	<i>halomonas eurihalina</i> F2-7	56.6%	96 hours	[62]
10	<i>candida lipolytica</i>	38%	72 hours	[63]
11	<i>saccharomyces cerevisiae</i>	83%	16 to 48 hours	[64]

12	<i>acinetobacter radioresistance</i> KA53	59%	3 to 5 days	[65]
13	<i>acinetobacter calcoaceticus</i> RAG-1	21%	120 hours	[66]
14	<i>bacillus subtilis</i>	45%	20 days	[67]
15	<i>candida lipolytica</i>	8%	8 to 16 hours	[64]

V. CONCLUSION

Removal of benzene from the subsurface environment is a crucial world problem. A better understanding of the mechanism of biodegradation and varieties of microorganisms involved in the clean up has a high ecological significance. It may depends on the indigenous microorganisms to transform or mineralize the organic hydrocarbons. Microbial degradation process helps the elimination of spilled benzene from the environment after critical removal of large amounts of it by various physical and chemical methods. This is possible because microorganisms have systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Therefore, based on the present review, it may be concluded that microbial degradation can be considered as a major component in the cleanup strategy for benzene remediation.

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