1. Introduction and literature review

1.1 History of Benzene

Michael Faraday was the first who invented the benzene in the year of 1825 using illuminating gas. Further the German scientist Eilhardt Mitscher lich heated up benzoic acid with lime to produced benzene in the year 1834. In the year of 1845, German chemist A.W. Von Hofmann successfully extracted pure benzene from coal tar. Benzene is also known as benzol which is a colorless liquid with a sweet smell, vaporized into atmosphere easily and moderately soluble in water. It is highly flammable in nature and found in air, water as well as soil. Various chemicals derived from benzene are shown in Fig.1. The permissible limit of benzene in water is 5µg/L (USEPA, 2009). Benzene is widely utilized as organic solvent in chemicals manufacturing industries. Both anthropogenic and natural sources are responsible for benzene in air, water and soil (ASTDR 2007).

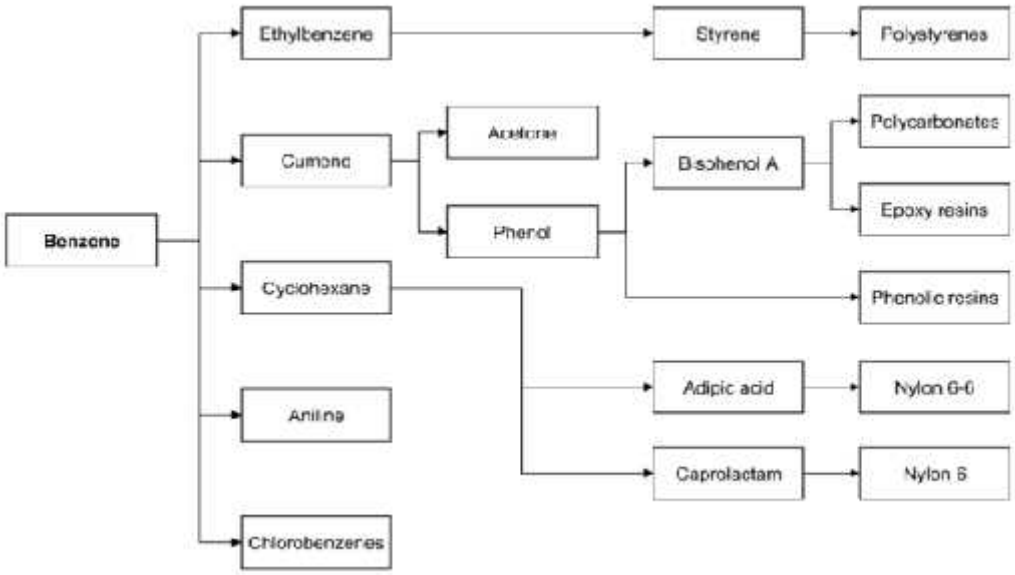

Fig.1 Major commodity chemical and polymer derived from benzene (USITC, 2003; ASTDR 2007)

1.2 Natural and industrial sources of benzene

The natural sources of benzene are volcanoes and forest fires which contribute to benzene in the atmosphere. Benzene is also produced from gasoline, crude oil and cigarette smoke (ASTDR, 2007). Benzene is mainly manufactured from petroleum products. Because of its widespread applications in industries, production wise it is ranked in the top 20 chemicals produced in the United States (USEPA, 2009; WHO 1997). The variety of industries use benzene to manufacture other chemicals including styrene, cumene, cyclohexane, rubbers, lubricants, dyes, detergents, drugs, pesticides etc. (ASTDR, 2007; WHO, 1997).

1.3 Exposure and toxicity of benzene in the environment

Benzene contamination of soil, water and air may be due to man made as well as natural processes. As increase the demand and application of benzene as a solvent in many manufacturing processes, it's concentration level rises in the environment. The major cause

of increased benzene level in the atmosphere is emissions of gases from burning oil and coal, benzene waste and motor vehicle exhaust etc. Similarly industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks release benzene into water and soil.

Toxicity of benzene increases in the water due to it's soluble nature. The source of benzene in the water bodies is the discharge of waste from industries, underground storage tank, leakage of pipeline, accidental spillage as shown in Fig. 2 which contaminates the nearby surface water, water bodies and environment. The benzene contamination of water affects the aquatic life, animals and human being. The permissible limit of benzene in a drinking water is 5µg/L (ASTDR 2007;USEPA, 2007; WHO, 1997).

Fig.2 The leakage of pipeline, accidental spillage of benzene (USEPA, 2007, 2009)

1.4 Health effect

Exposure of benzene associated with a range of adverse health effects as acute, long-term and short diseases, including cancer and a plastic anemia. Inhalation of high doses of benzene affects the nerve system which causes the symptoms like dizziness, drowsiness, headache, and unconsciousness etc. (ASTDR, 2007; WHO, 2010). Contaminated foods and fluids with high concentration of benzene consumed by human being can cause vomiting, stomach irritation, sleepiness, and rapid heart rate (WHO, 2010).

Long-term exposure of benzene mainly harms the bone marrow, inner parts of bones which results in Anemia, also affects the reproductive system in human being (affects the fertility in women, ovary shrinking and disturbed the menstrual cycle etc.) (WHO, 2010). Thus, continuous monitoring and remediation of benzene from the contaminated environment are an important issue. The conventional remediation techniques, such as thermal, extraction, steam stripping, chemical oxidation, adsorption, etc. may be used to reduce high concentrations of benzene and similar organic pollutants (Takahata, 2006; Padhi and Gokhale, 2016). However, these processes have several limitations such as high cost and generation of additional toxic products. Hence, there is a scope for a study on removal of benzene using a more reliable technique such as bioremediation which has been already proven economical and eco-friendly for the removal of pollutants from the contaminated environment (Singh et al., 2010; Kureel et al., 2016; Geed et al., 2017). Bioremediation involves the manipulation of environmental parameters to allow microbial growth and enhanced degradation of pollutants without any damage to the environment (Fulekar, 2005). Slow rate of degradation is the only major problem of bioremediation and most of the research work is going on to enhance the rate of degradation. The selection of suitable microorganisms, process optimization, selection of

good bioreactor systems with suitable packing material such as agro-waste, wood charcoal, PUF etc. may resulted in significant enhancement of rate of biodegradation. (Singh et al., 2006; Shukla et al., 2010; Singh et al., 2010; Kureel et al., 2016; Geed et al., 2017; Singh et al., 2017).

1.5 Bioremediation

Bioremediation define as "treatment technology that uses biological activity to reduce the concentration or toxicity of the pollutants. It commonly relies on the processes by which microorganisms transform or degrade the waste in the environment". The removal and mineralization of hazardous compounds from contaminated soil/water by the help of green plants (Phytoremediation), bacteria and fungi is defined as bioremediation process. Fungi and bacteria species break down the contaminants into less harmful substances. In bioremediation process, microbes produce specific enzymes which act as biocatalyst to oxidise or degrade the pollutant into harmless products through various metabolic pathways. In this process the pollutants are utilised by the bacteria as a source of energy and nutrients for their growth. The microorganisms (fungi and bacteria) used to bio-transform hazardous highly toxic pollutant to less toxic pollutant is not a new concept. Since 600 B.C. the application of microorganisms has been used by the human beings in different forms to treat organic pollutant. The commercial applications of bioreactor systems have been observed for the treatment of toxic pollutant since last 30 years. In 1972, Pennsylvania, Sun oil pipeline spillage in Ambler was used the first commercial application of bioremediation technology (National Research Council, 1993). After 1972, this technology has been well-developed to clean up various organic pollutants including BTEX. EPA (Environmental Protection Agency) of United States, conducted a survey in 1992 and provided information about 240 cases of bioremediation (Alexander, 1994). The

majority of the cases were reported to treat the organic pollutant in ground water as well as soil. The chemical and petrochemical industries in US drain their effluent in open area and river near by industries owing to this requirement of cost effective technology like bioremediation to treat organic pollutants. The choice of bioremediation is ecological and inexpensive technology and has two types' *in-situ* and *ex-situ*. The bioremediation is depending on operating process parameters like concentration, temperature, pH, potential microbes and bioreactor systems (Alexander, 1994; Dou et al., 2010).

The Fig.3 shows the biodegradation of petroleum products in the soil. In the metabolic pathway, the bacterial species first metabolise the petroleum products into naphthenic alcohols, acids, hydro peroxides, phenols, esters, carbonyl compounds, and finally to converted to water and carbon dioxide (Eglinnton, 1975; Markovi *et al*., 1996; Singh and Fulekar, 2010, Liu et al., 2010, Dou et al., 2010).

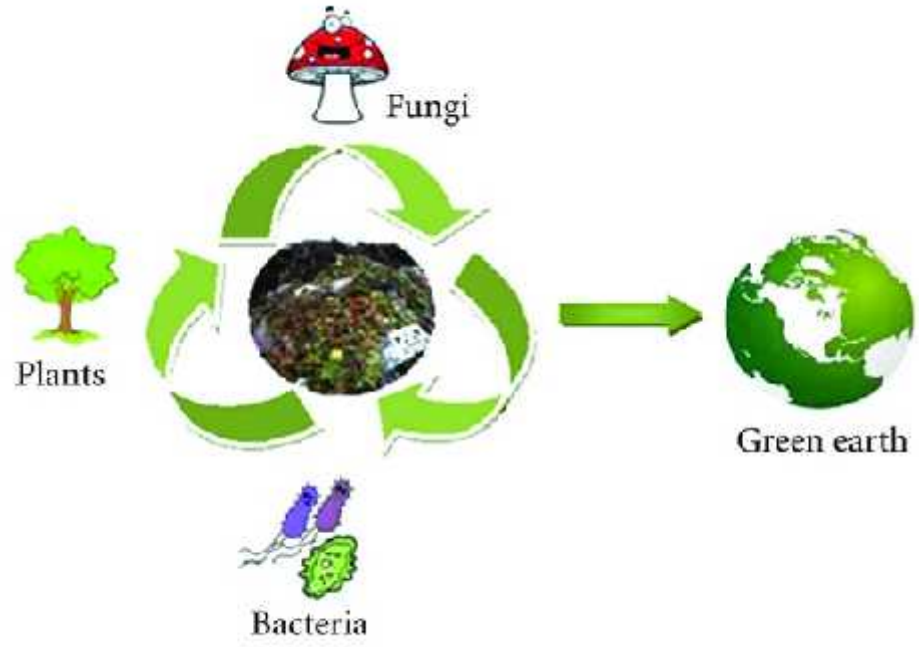

Fig. 3 Process of waste bioremediation (Karigar and Rao, 2011)

1.6 Bioremediation technology

Biostimulation, bioaugmentation, composting, bioventing, and land farming, are based on the principles of bioremediation technologies as shown in Fig.4. The selection of suitable technology for bioremediation depends on many factors such as nature of compound (difficult or easy to degrade), *in-situ* and *ex-situ* application, availability of bacteria (mixed or specific bacteria) etc. Singh et al. (2014) proposed two phase bio treatability assays for *In-situ* biotransformation of organic waste. In the first phase indigenous microbes were selected and their metabolic actions were studied along with existence of possible inhibitors and other inhibitory conditions. The process parameters were optimized to get the best results. In the second phase (Bento *et al*., 2005) more studies such as site-specific characterization of experimental work, change in soil parameters and change in indigenous soil microbial population were studied (Singh et al. (2014).

1.6.1 In-situ bioremediation

In-situ technique treats pollutant at the site and can be defined as the process in which organic waste are biodegraded under normal environmental conditions to water, carbon dioxide and metabolites. This technique has low maintenance, low-cost, sustainable approach and environment-friendly for the cleanup of contaminated area (Seech et al., 2008).

1.6.2 Types of in situ bioremediation

Bioattenuation is process in which natural degradation of pollutant occur at accelerated rate (Kanissery and Sims, 2011). **Biostimulation** is basically stimulation the ecological native capable bacteria for bioremediation. The stimulation can be achieved by

incorporation of different forms of electron acceptors and degradation rate limiting nutrients like nitrogen, oxygen, phosphorus, carbon etc (Kanissery and Sims, 2011).

Bioaugmentation is the addition of bacterial cultures required to speed up the rate of degradation of a contaminant. Organisms that originate from contaminated areas may already be able to break down waste, but perhaps inefficiently and slowly. Bioaugmentation usually requires studying the indigenous varieties present in the location to determine if biostimulation is possible. If the indigenous variety do not have the metabolic capability to perform the remediation process, exogenous varieties with such sophisticated pathways are introduced. This technology is generally used in municipal/industrial effluent treatment (M Tyagi, et al; 2011).

Bioventing: is the process in which oxygen is provided in the pollutant soil by movement of air in order to increase the oxygen concentration and also stimulate bioremediation

Biosparging is the aerobic process in which injection of air in to the ground water to improve the volatilization and bioremediation of organic pollutant.

1.7 Ex situ treatment processes

In ex-situ bioremediation excavate the soil from polluted site a place, aerated lined above the ground treatment level and stimulates the degradation of pollutant by potential microbes. The isolated potential microbes can utilize pollutant (cresols, phenols, polycyclic aromatic and petroleum hydrocarbon etc.) as an energy and carbon source to mineralize them into water, carbon dioxide and metabolites (Jorgensen, 2007).

Land treatment is the process in which contaminated sediment, sludge or soil is excavated placed lined beds, and turned over regularly at an interval of time to aerate the polluted area.

Composting is the biological decomposition of organic waste such as food or plant material by bacteria, fungi, worms and other organisms under controlled aerobic (occurring in the presence of oxygen) conditions. The end result of composting is an accumulation of partially decayed organic matter called humus.

Biopiles are an ex situ, solid-phase biological process for converting contaminants to low-toxicity byproducts. Biopiles are aerated with the use of perforated pipes and blowers in order to control the progression of biodegradation more efficiently by controlling the supply of oxygen.

In the bioremediation, selection of suitable microorganism is the most important factor for effective removal of pollutants. Specific microbes and consortia have been used for biodegradation of benzene in air (vapour) as well as liquid phase by various researchers (Singh and Fulekar, 2010; Robledo-Ortíz et al., 2011; Tsai et al., 2013).

However only few researchers have performed the removal of aqueous phase benzene using bacterial sp. like *Alcaligenes xylosoxidans*Y234, *Pseudomonas putida* MHF 7109, *Pseudomonas putidaP. fluorescens* ,*P. putida* F1,*Bacillus* sp. *Rhodococcus* sp in hybrid, two-phase partitioning and fibrous bed bioreactor (Banerjee and Gokhale 2016; Sung et al 1999,Yang et al 2001; Singh and Fulekar 2010; Tareq et al 2004; Pena and Ortiz et al 2008)

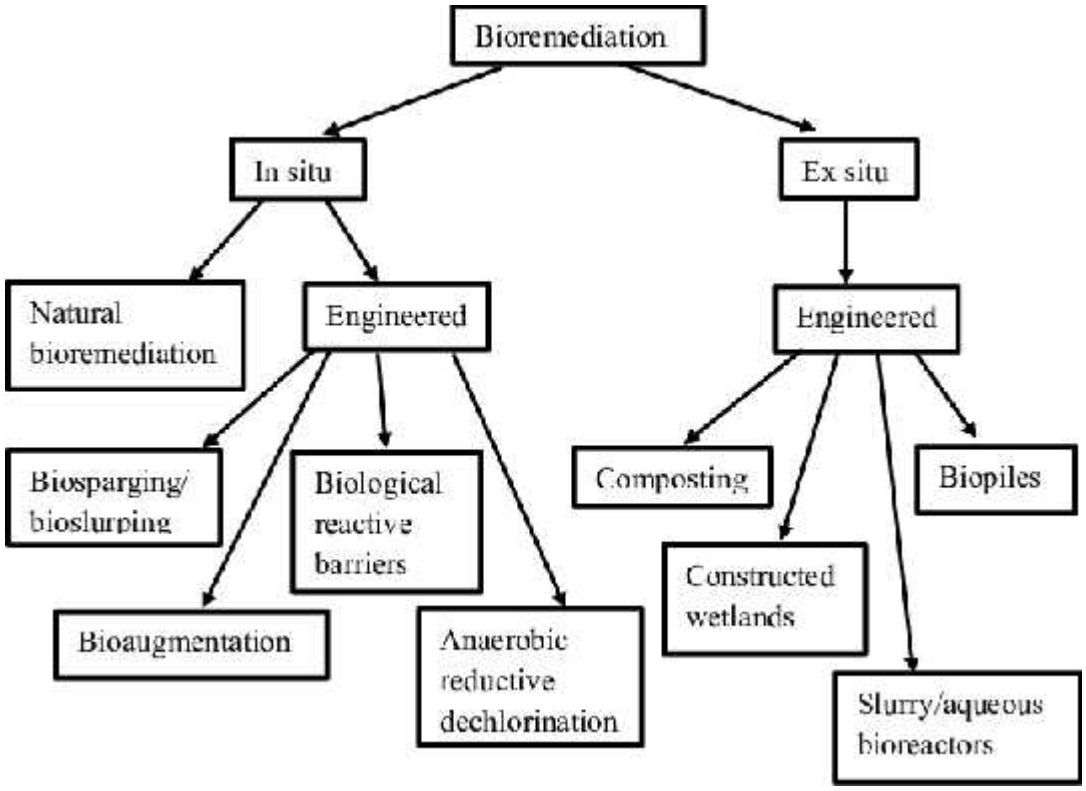

Fig.4 Bioremediation classification process (Oyetibo et al 2016)

1.8 Bioreactor used for the treatment of benzene

Selection of suitable bioreactor systems along with packing materials is another important aspect to enhance the rate of degradation. The several reactor systems such as a free cell, immobilized systems have been used by the researchers in their work. Free cell systems are simpler in nature but offer less degradation as compared to immobilized reactors (batch and continuous) so not suitable for real applications (Robledo-Ortíz et al., 2011; Kureel et al., 2016). However, experimental results of free cell system can be used for process optimization and design of immobilized reactors (batch and continuous). In the immobilized systems, selection of support media for immobilization of bacteria is an important factor which decides the performance of the bioreactor system. The various

researchers have used variety of bioreactor for the treatment of liquid benzene (Singh and Fulekar, 2010; Huang and Yang, 1998)

1.9 Types of the bioreactor

1.9.1 Fibrous bed bioreactor

The fibrous-bed bioreactor has previously been used to provide a novel cell immobilization process involving both attachment and entrapment that allows for continuous cell regeneration as shown in Fig 5, adaptation, and in-process selection of (mutant) strains suitable for the process purpose (Yang et al., 1994; Silva and Yang, 1995; Huang and Yang, 1998). The fibrous-bed bioreactor may also be used as a tool to obtain new microbial strains or mutants for treating chemical pollutants, such as benzene (Shim and Yang 1999).

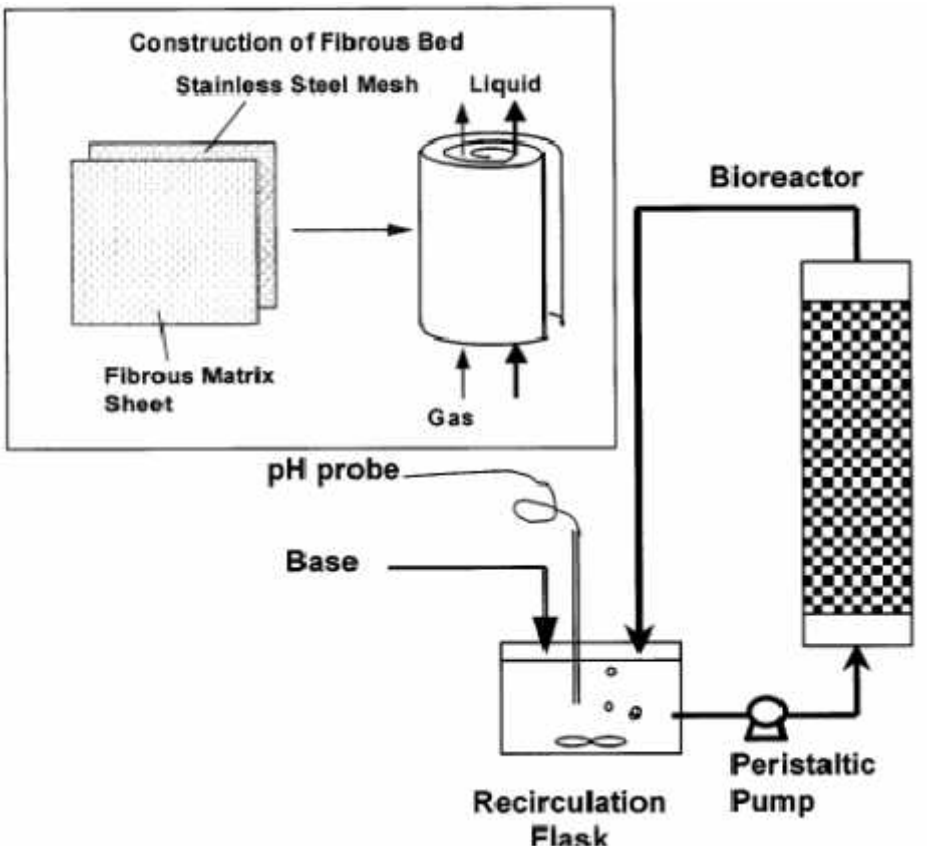

Fig 5 Fibrous bioreactor for treatment of aqueous benzene (Shim and Yang 1999)

1.9.2 Two-phase partitioning bioreactor (TPPB)

The TPPB concept is based on the use of a water immiscible and biocompatible organic solvent that is allowed to float on the surface of a cell-containing aqueous phase as shown in Fig.6. The solvent is used to dissolve large concentrations of benzene substrates into the aqueous phase. This is usually achievable because of the most organic contaminants are very hydrophobic in nature, which then partition into the aqueous phase at low levels. Thus, although very high amounts of toxic organic substrates can be added to a bioreactor, the cells experience only very low concentrations (Andrew J. Daugulis 2001; Singh and fulekar 2010).

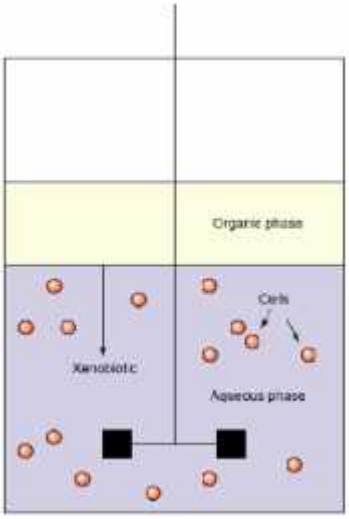

Fig.6 Two phase partitioning bioreactor for treatment of xenobiotics including aqueous benzene (Andrew J. Daugulis 2001)

1.10 Packing media used in bioreactor

The ability of bioreactor to remove pollutant largely depends on the selection of packing material used in the bioreactor. The packing medium described as solid support on which biofilm formation of microbes, which grows as a result of pollutant degradation.

The choice of media is crucial in order to maintain long-term efficient bioreactor operation. The desirable properties which are considered during the choice of the specific packing material are its density, specific surface area, water holding capacity, elemental composition, buffering capacity, pH, and porosity of the material (Bohn, 1996). Presence of these properties ensures intrinsically active bioreactor medium with large effective surface area and uniform distribution of air throughout the bed without channeling and gapping (Deshusses et al., 1995; Ergas et al., 1994).

The packing materials can be broadly classified into two categories i.e. natural and synthetic materials. Soil, compost, peat, wood chips, biomass, coal are the most commonly used as a natural packing materials and Pall rings, granular activated carbon (GAC), polyurethane foam, alginate bead etc. are commonly used synthetic media. Natural materials show compaction problem and may deteriorate over long term operations so some inert materials like poly vinyl alcohol, ceramic, glass beads, polypropylene (Chan and Lu, 2003; Chan and Lin, 2006), (Cardenas-Gonzalez et al., 1999; Song and Kinney, 2005; Kennes and Veiga, 2001; Rene et al., 2009; Krishnayya et al., 1999, Pineda et al., 2000; Ying et al., 2005 Chan and Lin, 2006) are blended with them to increase their strength. Nutrient supply is also a critical parameter for the microbial activity, so nutrients must be added during the operation. Synthetic composite materials containing macro and micro nutrients were also prepared and used as bioreactor packing in order to overcome the problem of continuous nutrient supply (Zilli et al., 2000, 2001).

1.10.1 Polyurethane foam (PUF)

Polyurethane foam has been found to be very useful by several researchers (Moe and Qi, 2002; Qi and Moe, 2006). For benzene removal study, polyurethane foam found to be superior packing over other types of packing materials used in the bioreactor. PUF is

mostly used because of it's desirable properties such as low density, high porosity, open structure, easy inoculation and good water absorb capacity etc. (Ryu et al. 2010; Yadav et al 2014). Ryu et al. (2010) used polyurethane bioreactor to study the relationships between biomass, and the performance of bioreactor under different loading conditions. Moe and Irvine (2000) used high density, open pore and self-manufactured polyurethane foam as bioreactor media and observed removal efficiency of more than 99% for 90 days.

1.10.2 PVA/Sodium alginate

Alginate beads was used as support medium for immobilization of bacteria due to their favorable physiochemical properties whereas excellent biocompatibility, presence of the microenvironment, and ability of cell confinement are some of the important properties of alginate beads and thus these materials are widely used by the researchers in different type of bioreactors (Patil et al., 2006; Mathur ea al 2010; Geed et al 2018). Mathur ea al (2010) successfully removed Cr(VI) by using calcium alginate beads. Similarly Banerjee and Ghoshal (2016) successfully degrade phenol using calcium alginate immobilized *Bacillus* cereus in a packed bead reactor. Mosmeri et al. (2017) reported the removal of benzene 100% at concentration (50 mg/L) in 4 days by using calcium peroxide (CaO_2) nano particles encapsulated in sodium alginate.

1.11 Affect of Processes Parameters like pH, Temperature, DO and nutrients supply on the bioremediation benzene in wastewater

In bioremediation process treat contaminated soil microbial species are present the indigenous microbe activity and growth must be stimulated by the addition of oxygen and nutrients. The basic need of microorganism is nutrients and carbon source and producing the essential enzymes to oxidize the pollutant. All of them will need phosphorous, carbon and nitrogen out of which carbon is the important element and required larger quantities

than other elements for living organism. In addition to carbon, nitrogen, oxygen and phosphorous are important constitutes required for successful biodegradation. The requirement of nutrients phosphorous to carbon is 1: 30 and nitrogen to carbon ratio is 1:10 (Vidali, 2001; Singh and Celin, 2010).

Temperature, pH, and DO affect the microbial growth. Optimum value of these parameters enhance the rate of degradation significantly. pH is an important parameter play crucial role in the metabolic pathway of bioremediation. pH in the bioreactors may be controlled by organic acids and bases. Biochemical reactions are highly sensitive to temperature and the rates of many bioremediation reactions are double for 10 °C increased in the temperature. In a certain temperature range the cells die and hence the plastic wrapping can be used to enhance the biodegradation in solar warming in late spring, autumn and summer. The oxygen availability for biodegradation decides whether the biodegradation process will be aerobic or anaerobic. Most of the hydrocarbons are easily biodegraded under aerobic state, while few hydrocarbons such as chlorinated compounds are more easily biodegraded in anaerobic condition. The oxygen in the bioreactor can be increased by various techniques such sparging of air. The air, water, and nutrients delivery is control by soil structure and composition (Vidali, 2001; Shukla et al., 2010; Yadav et al., 2014, Singh and Fulekar, 2010). The details of literature on Benzene were given in **Table1**

1.12 Literature review on bioremediation of benzene:

The bioremediation process has several advantages such as cost effective and environmental friendly in nature, convert the harmful waste into harmless products overall these processes are categorized as green processes but due to slow nature of the bioremediation processes the field applications are still limited. Therefore, the major

challenge in the bioremediation research is enhancing the rate of process and only then the large scale commercial application of this process will be feasible. For enhancing the rate of bioremediation the current research is mainly focused on isolation and selection of efficient microbial species, improved reactor configuration, optimization of process parameters and hybrid bioreactors which combine the advantages of bioremediation as well as established conventional processes. A representative summary of the research efforts in the area of bioremediation of benzene in particular and organic contaminant in general is presented in Table 1.

For bioremediation, the choice of potential microbes is most significant factor for removal of organic pollutants including benzene. The mixed cultures as well as specific microorganisms have been used for the degradation of organic pollutants were performed by various researchers as shown in Table 1. The specific microbial species which have been isolated and tested for biodegradation of benzene and shown promising results are *Janibacter sp. SB2, Azoarcus and Georgfuchsia, Bacillus sphaericus, Mycobacterium sp., P mendocina,RalstoniapicKettii,Burkholderiacepacia,Rhodoccusrhodochrous, P. fluorescens, Psudomonas putida* out of which *Psudomonas putida* was found most common bacterial species reported by different researchers. Some researchers have also used mixed consortia (Chi-HuiYeha et al.2010, Firmino Paulo Igor M. et al. 2015, and Simantiraki et al. 2013) and Fungi (Garca Pena et al. 2008). The performance of bioreactor enhances significantly using good microorganism but further enhancement is only possible by selecting good reactor system along with optimized process parameters .Researchers have used different types of reactors such as batch with free cell system, immobilized batch, continuous free cell and immobilized continuous reactors. Batch free cell reactors are most common (Chi-HuiYeha et al., 2010; Tarık et al., 2004; Gunaseelan et al., 2003; Yu et al. 2001; Reardon

et al., 2000; Kim et al., 2005; Chang et al., 2017; Lin, 2012; Garca Pena et al., 2008; Sebastian Stasik et al., 2015; Martin Sperfeld et al., 2018; Hyun Mi Jin et al., 2013; Simantiraki et al., 2013; Padhi and Gokhale, 2017; Mosmeri et al., 2017; Liu et al., 2010). Some researchers have also evaluated batch immobilized reactors (Singh and Fulekarl., 2010; Robledo-Ortíz et al., 2011; Tsai SL et al., 2013; Tsai SL et al., 2013; Hojae Shim et al., 1999; Jiin-Shuh Jean et al., 2008). Degradation in immobilized system was found better in general keeping other parameters same (Robledo-Ortíz et al., 2011; Kureel et al., 2016). The batch systems are simple in nature, cost effective, easy to operate but suitable for real time applications.

Limited studies are available on bioremediation of benzene in continuous immobilized systems such as two-phase partitioning, USAB, fibrous bed and hybrid bioreactor etc. (Firmino Paulo Igor M. et al 2015; Rahul, A.K.et al. 2013; Bielefeldt et al 1998; Bao-Ping Xin et al. 2013).These systems have shown better rate however have several limitations like a cell in excess of growth, striping difficulties to maintain the consistent aeration, etc.

Optimization of process parameters for improving the biodegradation is another important factor and helped to improve the overall bioreactor performance. Temperature, dissolved oxygen, concentration of substrate, pH, support media etc. were found important factors responsible for bioreactor performance. Good improvement in the bioreactor performance was observed after process optimization. Kinetic study (Robledo-Ortíz et al., 2011; Tsai SL et al., 2013; Hojae Shim et al., 1999; Tarık et al., 2004 ; Gunaseelan et al., 2003) helped us to calculate the bioreactor rate constants and the rate expression which is very useful for modeling and analysis of bioreactors mathematically. Kinetic study also helped to quantify

the efficacy of bacterial species and identification of inhibitory conditions in the bioreactors.

Table.1Literature overview on bioremediation of Benzene in aqueous phase

Bacterial species	Reactor type	Pollutants in liquid phase	Operating Conditions				Kinetics		Removal	References
			Concentration (mg/L)	pH	Time (hr)	Temperature (oC)	Ks (mg/l)	μ_{max}		
Psudomonas putida	Batch (immobilized system)	Benzene	15-90	7.0	11(free cell), 9h(immobilized system),	150 rpm, 30 ^{0}c	10.11, 10.80, -	μ_{max} 0.50 (1/h), 0.58(1/h),	100%	Robledo-Ortíz et al. (2011)
Pseudomonasputida	Immobilized system cow dung (Tow phase partitioning)	Benzene	50 – 250	-	24, 96, 168	25^{0}c, 100 rpm	-	-	65%	Singh and Fulekarl. (2010)
Mixed microbes	Free cell (Permeable reactive barrier reactor)	Benzene	20-320	-		150 rpm, 30±1 ^{0}c.	-	-	-	Chi-HuiYeha et al. (2010)
Pseudomonas species	Batch immobilized PVA-alginate Beads	Benzene	(17.6–27mg/l)		19, 49	30 ^{0}C 150 rpm.	4.93,	, 0.1155 (1/h),	99%	Tsai SL et al. (2013)
Pseudomonas putida; P. fluorescens	Batch, fibrous-bed bioreactor (immobilized),	Benzene	135mg/l (free cell), 1127mg/l (immobilized system)	7.0	---	25°C, 110 rpm	-	0.88(1/h), 0.92 (1/h)	-	Hojae Shim et al.(1999)

Pseudomonas sp.	Batch, fibrous-bed bioreactor(immobilized),	Benzene	-	7.0–7.5	60	20-28 ^{0}C,	-	-	-	Jiin-Shuh Jean et al. (2008)
Pseudomonas putida F1 ATCC 700007 (Pp F1)	Batch (free cell),	Benzene	187.7mg/l,	7.0	24	30 ^{0}C,	1.65	$\mu_{max,}$ =0.62,	-	Tarık et al. (2004)
Rhodoccusrhodochrous	Batch,	Benzene	(0-80mg/l)	6.8-7.0		35 ^{0}C,	-	1.2mg/mg per day	-	Deeb et al. (1999)
Pseudomonas putida F1, P. putida mt2, P. mendocina Ralstoniapickettii Burkholderiacepacia	Batch (free cell)	Benzene	50mg/l	-	-	20°C	0.7-1.3	0.35-0.37,	-	Gunaseelan et al. (2003)
Agrobacterium sp.,	Batch (free cell)	Benzene	10-500 mg/l		48	150 rpm (28-30) ^{o}C	-	-	61.66 %	Yu et al. (2001)
Pseudomonas putida F1	Batch (free cell)	Benzene	43mg/l,	6.7–6.9.	-	200 rpm, 30°C	0.12 ± 0.02,	μ_{max} =0.73 ± 0.03,	-	Reardon et al. (2000)
Pseudomonas sp.	Batch (free cell)	Benzene	30-400mg/l	-		-	4.5	0.14	-	Kim et al (2005)

Pseudomonas sp. YATO411 Mycobacterium sp.	Batch	Benzene	120 mg/L	7.5	-	-	-	-	-	Chang et al (2017)
Pseudomonas sp. YATO411; Mycobacterium sp.	Batch	Benzene	(20–120 mg/L	-	-	28–30 _C.	-	-	67%	Lin (2012)
Consortia	UASB	Benzene	18mg/l	7.0		27 _C.	-	-	86%	Firmino Paulo Igor M. et al 2015
Mycobacterium sp. CHXY119; Pseudomonas sp. YATO411	Bio-PRB	Benzene	100 mg /L	7.0		25 ◦C at 150 rpm			97.8%	Bao-Ping Xin et al. 2013
Bacillus sphaericus	Continuous Bench Scale corn cob-based biofilter	Benzene	0.0970 mg/l			30 ± 2 ◦C			> 99.85%	Rahul, A.K.et al. 2013
Free Fungus *Paecilomyces variotii* CBS115145	Batch (shake flask)	Benzene	30–60 mg / L	7.5		30 ◦C	-	-	100%	Garca –Pena et al 2008
Filamentous bacteria	Continuous shallow, sparged bioreactor	BTEX	2.3–4.3 mg / L	6.4–7.2		22–25 ◦C			> 97%	Bielefeldt et al 1998

Consortia	Batch	BTEX	1800 and 2100µg kg/L	7.0		-	-	-	70–90%	Sebastian Stasik et al 2015
Azoarcus and Georgfuchsia	Batch	Benzene	-	7.5	-	24 ^{0}C	-	-	-	Martin Sperfeld et al 2018
Janibacter sp. SB2,	Batch	Benzene	240 mg/L	7.0		100 rpm 25^{0}C 180 rpm	-	-	45.5%	Hyun Mi Jin et al (2013)
Consortia	Batch	Benzene	10mg/L	7.0		500rpm 20 ^{0}C	-	-	90%	Simantiraki et al (2013)
mixed microbial culture	Batch	Benzene	50-600mg/l	7.05		-	-	-	70%	Padhi and Gokhale (2017)
encapsulated calcium peroxide (CaO 2) nanoparticles	Batch	Benzene	50mg/l	3		30^{0}C	-	-	100%	Mosmeri et al. (2017)
Bacillus sp	Batch	Benzene	60-160mg/l	-		30^{0}C	-	-	92%	Liu et al (2010)

Findings of the literature survey

- Most of the researchers have performed their experiments in free cell batch systems which are not suitable for real time applications. However, these studies helped the researchers to establish bioremediation as proven environmental friendly alternative of other processes to degrade organic waste.
- Some researchers used immobilized batch system for biodegradation of benzene and observed enhancement in the performance.
- The process parameter optimization further helped to enhance the rate of degradation in the batch processes.
- On the basis of results batch studies few researchers tried degradation of benzene in continuous bioreactors however very limited and insufficient research work available in this area. So there is scope of research work on degradation of benzene in continuous bioreactors.
- Other areas where there is scope of research work are use of specific microorganism for biodegradation of benzene, use of efficient packing media in the Packed Bed Bioreactor for benzene degradation, kinetic study including inhibition kinetics, identification of metabolites in the degradation pathway of benzene, identification of enzymes (proteomic study) in the degradation of benzene and possibility of hybrid bioreactor in which conventional systems will be used to overcome limitations of bioreactors.

Research Objective

In the present it has been planned to study the biodegradation of benzene in batch as well as continuous bioreactors using the specific and efficient microorganism isolated from benzene contaminated site. On the basis of detailed literature review the following objectives were formulated for present study

- To isolate the efficient microbial species from benzene contaminated site.
- To evaluate the performance of immobilized batch system at different benzene concentration. also investigate the kinetic of degradation and to calculate the kinetic parameters including inhibition constant using available kinetic models and compared with experimental results
- To study the biodegradation of benzene in continuous packed bed bioreactor and to evaluate the performance of at varying loading rates under optimum condition.
- To identify and study the role of different enzymes (Proteomic study) produced during biodegradation of benzene.