Comprehensive study on reduction of toxicity of pollutants by microbial transformation

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Abstract- *As the world is becoming more advanced in the field of technology and mankind is backing intensively on various resources like petroleum, metal mines and non-renewable carbon sources, thus the environment is getting devastated by the excessive generation of toxic compounds, either as waste products or by-products. Quite a large number of microbes have the ability to transform these toxic pollutants into easily degradable reduced forms. This review basically aims at tabulating principal characteristics of various microorganisms which possess affinity towards toxic substances based on a number of biotic and abiotic factors like pH, temperature, functional groups, competition and moisture content. It classifies aerobic and anaerobic microbes on the basis of their role in biodegradation of organic, inorganic or radioactive pollutants and summarises different microbes used for reducing major soil, water and petroleum pollutants. Thereafter, it focusses on the bioreactors used majorly for in-situ and ex-situ bioremediation. It finally gives an idea of the prime factors which govern the rate of degradation, knowledge of different metabolic and enzymatic pathways which together can further be used to genetically engineer new strains to effectively reduce various other recalcitrants for a sustainable future.*

Keywords- *Pollutants, Reduction, Aerobic, Anaerobi, Microbes, Transformation, Bioremediation, Bioreactors.*

I. INTRODUCTION

Microbial transformations use the microbes to clean up polluted water and soil. Microbes are small organisms, like fungi, bacteria which are omnipresent. Microbes use pollutants as their source of energy and the end products of the source are the reduced form of pollutants that can be degraded easily also known as Microbial transformation. Predominantly petroleum by-products, solvents, oils and pesticides are the pollutants that are treated by using microbial transformation. The transformed products are harmless substances such as small amount of water and gases like ethane and carbon dioxide. By bioaugmentation, the microorganisms which lead to transformation of pollutants can be added to the groundwater and soil to bring about the reduced form of pollutants. The efficiency of bioremediation depends on various factors like ambient temperature, food and nutrients present, as they help the required microbes to proliferate rapidly and degrade pollutants more effectively. Under unsuitable conditions the survival of the microbes is hampered and the pollutants don't get degraded completely making the process inefficient. Amendments are done to the contaminated soil and ground water to improve the conditions for the growth and multiplication of the required microbes. The different kinds of amendments include the addition of vegetable oils, molasses, chemicals that results in enhancing the level of oxygen. These amendments are sometimes pumped in underground wells to treat both contaminated soil and water.

The duration of microbial transformation varies from few days to years as it takes time for the microbes to act on a particular site. Microbial transformation makes use of natural processes to clean up polluted areas. As it does not require much labour, energy as compared to other processes, it is much cheaper. Another added advantage of microbial transformation is that the transportation cost is saved as the contaminants are treated on the site itself. Bioremediation and be used to degrade pollutants originating from various sources, such as water, soil, industrial and others. Various bioreactors can be used for transformation of pollutants but membrane bioreactors and slurry bioreactors are chiefly employed for the transformation of waste water pollutants and contaminated soil respectively.

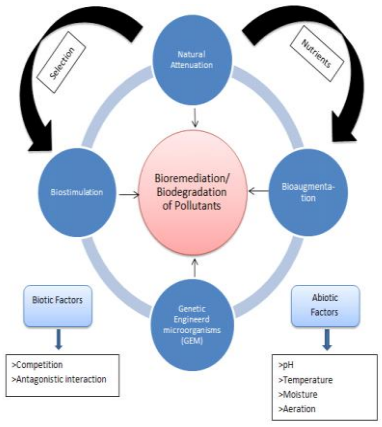

Fig 1 Flow chart explaining the cycle of bidegradation/bioremediation of pollutants.

II. CLASSIFICATON OF MICROBIAL TRANSFORMATION

To bring out microbial transformation, the physical and chemical characteristics of the pollutants of interest have to be known. The different environmental conditions required for promoting the growth as well as the proliferation of the required organisms have to be reviewed properly. If required, the environmental conditions have to be altered in order to establish favourable conditions for the organisms to carry out transformation.

Mainly there are two different types of bioremediation processes:

A. In-Situ Bioremediation: In –Situ Bioremediation mainly occurs at the site itself where the contaminant is present already. The pollutants are not transported from their original site. If the indigenous microbes are not present at the site then a consortium of required microbes are taken and added to the contaminated site. Majorly organic and aromatic pollutants are transformed in-situ.

B. Ex-Situ Bioremediation: Ex- Situ Bioremediation occurs when the contaminants are transported to some other place rather than transformation occurring at the place where contaminants are present. Transport of pollutant occurs from its original site to a preferred site where a consortium or a particular type of microbe is added to bring microbial transformation of the pollutants present in either water or soil. Chief ex-situ bio-remediated pollutants are hydrocarbons from crude petroleum and other organic pollutants.

The presence or absence of oxygen plays an important factor in microbial transformation. If oxygen acts electron acceptor then the process is called aerobic bioremediation while all other processes are called anaerobic degradation. Majorly one terminal electron acceptor is used by the microbes in most cases however some makes use of different electron acceptors. Facultative aerobes mainly use oxygen but switches to nitrate in the absence of oxygen.

The classification of bacterial metabolism can be seen in Table no.I

TABLE I
Classification of Bacterial Metabolism

AEROBIC	ANAEROBIC
Oxidation Co-metabolism	Denitrification Manganese Reduction Iron Reduction Sulfate Reduction Methanogenesis

III. CLASSIFICATION OF POLLUTANTS

Various hazardous pollutants are released in the environment in large amounts either accidentally (e.g. benzene, chlorinated solvents) or are released intentionally in huge amounts (e.g. pesticides). Among the variety of toxic pollutants released, there are also some types that are formed in very small quantity (e.g. dioxins). These pollutants are majorly present in soil and water and are either organic or inorganic in nature as has been tabulated in Table no.II.

TABLE II.
Classification of Different Types of Pollutants

POLLUTANTS	TYPE OF POLLUTANTS	SOURCE	NATURE
Trichlorobenzene (TCB)	Soil and Water	Industrial	Organic
Poycyclic Aromatic Hydrocarbon (PAH) [1]	Soil and Water	Industrial fuels/ combustion	Organic
2-Aminobenzoate	Water	Chemical industries	Organic
Diethyl phthalate	Water	Agricultural & Chemical industries	Organic
2,4-chlorophenoxyacetic acid butoxyethyl ester (DBE)	Water	Agricultural & Chemical industries	Organic
p-cresol	Water and Soil	Industrial	Organic
Phthalate esters	Water	Chemical industries	Organic
Vinyl Chloride	Groundwater	Industries	Organic
p- Nitrophenol	Soil	Fertilizer industries	Organic
3-Chlorobenzoate	Soil and Water	Chemical industries	Organic
1,2- Dibromoethane	Soil and Groundwater	Industrial	Organic

Bromophenol	Water	Chemical Industries	Organic
Carbon Tetrachloride	Soil and Water	Chemical industries	Organic
Polychlorobiphenyls (PCBs)	Soil	Industrial	Organic
Azo dyes	Soil and Water	Textile Industries	Organic
Chromate (VI)	Water	Industrial	Inorganic
N-Nitrosodimethylamine(NDMA)	Water	Industrial	Organic
Weathered Diesel Oils	Water and Soil	Petroleum Industries	Organic
Arsenite (III)	Groundwater	Industrial	Inorganic
Uranium and other heavy Metal	Groundwater	Radionuclide Industries	Radionudionuclides
Toxic SeO_3^{2-}	Groundwater and Soil	Hydrothermal Industries	Inorganic
Petroleum Hydrocarbons	Water	Petroleum Industries	Organic
Hexachlorobenzene	Water and Soil	Pesticide Industries	Organic
Lead	Soil	Chemical Industries	Inorganic
1,2,3-trichloropropane (TCP)	Groundwater	Industrial	Organic
Decabromodiphenyl Ether	Water and Soil	Industrial	Organic
Phosphates	Water and Soil	Agricultural and Chemical Industries	Inorganic

As it can be seen from Table no.II, many of soil pollutants and water pollutants come from sources like chemical industries, agricultural industries, pesticide industries, petroleum industries, hydrothermal industries, radionuclide industries, textile industries, fertilizer industries and many other industries.

Some of the organic pollutants that are released in nature are Trichlorobenzene(TCB), Poycyclic Aromatic Hydrocarbon (PAH), 2-Aminobenzoate, Diethyl phthalate, 2,4- dichlorophenoxyacetic acid butoxyethyl ester (DBE), p-cresols, Phthalate Esters, Vinyl Chloride, Carbon Tetrachloride etc. Whereas the list of inorganic pollutants includes Chromate (VI), Arsenite (III), Toxic SeO_3^{2-}, Mercuric Ion (Hg (II)), Lead, Phosphates etc. Some of these pollutants are present in both soil and water and comes from various sources that have been mentioned before. It was seen that radionuclide pollutants such as Uranium and other heavy metals which are also present in ground water can be transformed by microbes.

IV. MAJOR SOIL POLLUTANTS

The major soil pollutants are p-nitro phenol and xenobiotics (including polychlorobiphenyls (PCBs), polychlorodioxins, trinitrotoluene (TNT) and azodyes).

A. Nitrophenols: It was observed from the various studies that nitrophenols, falling under nitro aromatic compounds accumulates in the soil which is actually a result of hydrolysis of variety of organophosphorous insecticides like parathion or in the form of nitrophenols (usually herbicides) [2, 3]. The bacterias responsible for the degradation of p-nitrophenol were isolated from the soil namely *Arthrobacteraurescens* TW17 and *Nocardia sp*. Strain TW2. PNP degradation by *A.aurescens* TW17 was seen to be induced by pre-exposure to PNP (p-nitrophenol), 4 nitrocatechol, 4 nitrophenol or m-nitrophenol. [4]The same was done by *Nocardiasp* strain TW2 by PNP, 4- nitrocatechol, Phenol, m-nitrophenol or p-cresol. Finally, PNP was degraded to hydroquinone and nitrite by *A.aurescens* TW17 and the same PNP was transformed to 4 nitro-catechol by *Nocardia sp*. Strain TW2 [4].

B. Xenobiotics: The studies related to major xenobiotic compounds like PCBs, TNTs, polychlorodioxins and azodyes have shown that majority of the aromatic compounds available in the environment can be used by microorganisms as their sole source for carbon and energy [41]. Both aerobic (few like *Pseudomonas)* and anaerobic (*Azoarcus, Geobacter, Desulfobacterium, Methanospirillum*) microbes were used. In anoxic environment- the electron acceptors that were mainly used included, nitrate (denitrifying organisms), sulphate(sulphate reducers), Fe(II) (ferric oxide reducers), CO_2 (methano-

gens), or other acceptors (chlorate, Mn, Cr, U, etc) [5].

TABLE-III
Pollutants Transformed By Different Microbes

POLLUTLUTANTS	AEROBIC/ANAEROBIC	TRANSFORMED BY
1,2,3 and 1,2,4 Trichlorobenzenes (TCB)	Aerobic	*Pseudomonas putida*
2-Aminobenzoate	Anaerobic	Gram negative bacterias- *Pseudomonas* KB740 and KB820
Diethyl Phthalate	Aerobic	*Brevibacterium sp.*
2,4- Dichlorophenoxyacetic acid butoxyethyl ester	Aerobic	*Brevibacterium sp.* , *Chromobacterium sp.* and *Serratia sp.*
p-Cresol	Anaerobic	*Rivulariaceae*, *Tolypothrix*, *Anabaena*, *Chroococcus* and *Oscillatoria spp.*
Phthalate esters	Anaerobic	Phthalate degrading bacterias- strain CC9M, PP-1 and OP-1
Vinyl chloride	Aerobic	*Aspergillusfumigatus*, *Phanerochaetechrysosporium*, *Lentinustigrinus*, *Aspergillusniger*
p-Nitrophenol	Aerobic/ Facultative anaerobic	*Arthrobacter aurescensTW17 and Nocardia sp. TW2*
Recalcitrant trace metals	Aerobic	*Pseudomonas atlantica*, *P.aerogenosa*, *Pseudomonas*
2,4 Dichlorophenoxyacetate		*Burkholderiacepacia*, *Pseudomonas putida*, *Arthrobacter sp.*, *and Sphingomonas sp.*[6, 7]
1,2- Dibrobromoethane	Aerobic	*Xanthobacterautotrophicus GJ10*and*Ancylobacteraquaticus*[8]
Bromophenols	Anaerobic	Sulphate reducing bacteria and iron reducing bacteria [9]
Carbon tetrachloride	Anerobic	Acetogenic and methanogenic bacteria
Toxic Hydrocarbons	Anaerobic	*Nitrosomonaseuropa*, *proteobacter*,*nitrobacter* [10]
Napthalene	Anaerobic	Napthalene degrading pure cultures, strains- NAP-3-1, NAP-3-2 and NAP-4
Xenobiotics- PCBs, Polychlorodioxins, Trinitrotoluene and azo dyes	Aerobic/ Anaerobic	Aerobic- *Pseudomonas* Anaerobic- *Azoarcus*, *Geobacter*, *Desulfobacterium*, *Methanospirillum*
Chromate (VI)	Aerobic	*Pleurotus*, *Bjerkandera*, *Phanerochaete and Trametes [11]*
Polycyclic Aromatic Hydrocarbons (PAH)	Aerobic	*Bacillus subtilis* [12]
N-Nitrosodimethylamine	Aerobic	*Pseudomonas mendocina KR1*
Weathered diesel oils	Facultative Anaerobes	*Staphylococcus hominis*, *Kocuriapalustris*,*Pseudomonasaerogenosa* , *LBI*, *Ochrobactrumanthropi and bacillus cereus* [13]
Arsenite (III)	Aerobic	*Pseudomonas*, *Alcaligenes*, *Thiomonas*, *Herminiimonas*, *Agrobacterium* and *Thermus*
Uranium and other heavy metals	Anaerobic	*Desulfobacterales&Desulfovibrionales*, *Syntrophobacteraceae and Clostridiales*
Toxic SeO_3^{2-}	Aerobic	*Bacillus cereus* CM100B
Petroleum Hydrocarbons	Aerobic/ Anaerobic	*Pseudomonas sp.*, *Bacillus sp.*, *Alcaligenes sp.*, *Corynebacterium sp.*,
Penta-	Aerobic	Synthetic microbial

chlorophenol (PCP)		communities-*Sphingobium-chlorophenolicum* (in the core layer) and *Ralstoniametallidurans* (Hg(II) reducer)[14]
Hexachlorobenzene (HCB)	Aerobic	*Dehalococcoides sp. Strain CBDB1* and*dehalococcoidesethenogens*strain 195and *aDehalobiumchlorocoerciaDF*-1 [15]
Lead	Aerobic/ Anaerobic	*Metrahiziumanisopliae, Paecilomycesjavanicus*and *Aspergillusniger*[16]
1,2,3-trichloropropane (TCP)	Anaerobic	*Dehalogenimonaslykanthroporepellens*and *Methylosinustrichosporium*[17]
1,3-dichloprop-1-ene	Aerobic	*Pseudomonas pavonaceae*
Decabromodiphenyl Ether	Anaerobic	*Geobacter, Shewenalla*, and *Pseudomonas*[18]
Phosphates	Anaerobic (Majorly)/ Aerobic	*Dechloromonas, Acinetobacter, Zoogloea*[19]

V. MAJOR WATER POLLUTANTS

The major pollutants that increases the toxicity of water are 2-Aminobenzoate, Diethyl phthalate, 2,4-dichlorophenoxyacetic acid butoxyethyl ester (DBE), Phthalate esters, Vinyl chloride, Bromophenol, Chromate (VI) , N-Nitrosodimethyamine (NDMA), Arsenite (III), Uranium, Petroleum Hydrocarbons, 1,2,3-Trichloropropane (TCP) etc.

A. 2-Aminobenzoate: The niro and aminoniroaromatic compounds are produced in huge quantities by the chemical industries. Mainly these are xenobiotic compounds that can cause severe environmental pollution. When O_2 is present many aminoaromatic compounds polymerize to recalcitrant molecules. To prevent the formation of these molecules an anaerobic biodegradation of a single class of compound was carried out. Two strains of bacteria belonging to the gram negative bacteria family, *Pseudomonas*- KB740 and KB820 were isolated. These strains were known for the degradation of aminobenzoate anaerobically with nitrate as the terminal electron acceptor under denitrifying conditions[20]. 2-Aminobenzoate was oxidised to CO_2 and NH_4 and nitrite was reduced to N_2.

B. Diethyl phthalate and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (DBE): The studies mainly focussed on the culture filtrates ,mixed populations and effects of standard microbial exudates on the bacterial transformation. *Zygomycetes* and *Ascomycetes*wereproven to inhibit the growth of microbes. The microbes used were *Flavobacteriumaquatile* and *Flavobacterium sp*. which reduced 2,4-DBE. *Chromobacteriumsp, Brevibacteriumsp*and *Serratia sp.* transformed 2,4-DBE, both 2,4-DBE ; DEP and only 2,4-DBE respectively[21].

C. Phthalate esters: These are the industrial chemicals that are produced in large amount as they are used as plasticizers[22, 23]. Bacteria were isolated from marine sediments that proliferated anaerobically on m-phthalate, p-phthalate or dipicolinate [2,6 PDCA]. Intact cells of each organism showed Na^+ dependent oxidation of their growth substrates. Pure cultures of marine isolates were grown on m or p-phthalate partially metabolized the appropriate structural PDCA but a marine bacterium that grew on 2,6- PDCA did not metabolize m-phthalate. It was observed that the enzymes involved in the hydroxylation of aromatic compounds contribute to the degradation of the pyridine derivatives. 2,6 PDCA was transformed by the strain CC9M when this strain was allowed to grow on m-phthalate; 2,5 PDCA was metabolized by strain PP-1 when grown on p-phthalate whereas 2,3 PDCA was oxidised by strain grown OP-1 grown on o-Phthalate[24].

D. Vinyl chloride: In various studies it was seen that vinyl chloride's relatively high aqueous solubility and persistence in soil was responsible for the increase of chlorinated aliphatic hydrocarbons in groundwater and it is toxic and carcinogenic to humans. Under Aerobic condition, Vinyl Chloride can be readily reduced. More than 99% of the labelled material was degraded after 108 days and approximately 65% being mineralized to $^{14}CO_2$. Biotransformationn under methanogenic conditions lead to slow and incomplete degradation but under aerobic conditions it was rapidly degraded. The ability of microbes associated with soil and groundwater to degrade vinyl chloride is wide spread[25].

E. Bromophenol: The marine ecologies are a rich source of naturally occurring halogenated compounds, which includes bomophenols . Therefore it is highly likely that microorganisms existing in these environments have developed the ability to utilize these halogenated compounds. Studies revealed that presence of various electron acceptors is a characteristic of anaerobic biodegradation. It

was observed that 2-bromophenol was debrominated and reduced to phenol. 9-bromophenol and 4-bromophenol were monitored to get converted under few conditions which were sulfidogenic and methanogenic but did not get reduced under iron reducing conditions and it was examined in bromobenzoate that no intermediates were produced in the degrading cultures. Both sulphate reducing bacteria as well as iron reducing bacteria have been shown to use aromatic compounds chiefly including phenol as growth substrates[9].

F. Chromate (VI): Different redox enzymes are usually used to reduce the highly toxic compounds into less toxic compounds. One of the research conducted proclaimed the ability of chromate reductases to carry out the reduction of highly toxic Cr(VI) to a much less toxic insoluble compound Cr(III). Chromate(VI) is a by-product of numerous industrial processes such as leather tanning, pigment production, chrome-plating and thermonuclear weapon manufacture. Due to its rapid leaching capability, it contaminates drinking water supplies. The microbes used in the transformation were mainly found to be*Pleurotus, Bjerkandera, Phanerochaete and Trametes* [11].

G. N-Nitrosodimethyamine (NDMA): One of the prime studies focussed on to elucidate the pathway(s) of NDMA biotransformation by *Pseudomonas mendocina KR1*, which is a strain possessing enzyme toluene-4-monooxygenase (T4MO). NDMA is usually present as a by-product of wastewater and drinking waterdisinfection and disposal of 1,1- dimethylhydrazine. Initially NDMA was found to get oxidized to nitrodimethylamine (NTDMA) and further oxidized to N-nitromethylamine .The strains incubated with NDMA also resulted in production of minute concentration of methanol (CH_3OH) [26].

H. Arsenite: Lately the chronic consumption of groundwater with high arsenic levels has led to endemic arsenicosis spread across China and several new cases of arsenicosis are appearing at a fast rate [27] .Bacteria have developed unique abilities to reduce arsenic majorly by arsenite oxidation, respiratory arsenate reduction, cytoplasmic arsenate reduction, and arsenite methylation [28]. Some of these transformations primarily aim at reducing the arsenic toxicity. Arsenite-oxidizing bacteria aid in oxidizing arsenite [As(III)] to arsenate [As(V)] which is mostly considered a detoxification metabolism, As(V) being quite less toxic than As(III). As(V) can be easily absorbed as it is negatively charged, thus suchsuitable bacteria have been used in batch reactors together with immobilizing material for removing the arsenic from waste water [29, 30] .As(III) oxidation is prevalent in numerous bacteria including *Pseudomonas*, *Thiomonas*[31], *Herminiimonas*[32], *Alcaligene* [33], *Agrobacterium* , and *Thermus*. Some of these bacteria grew as lithotrophs and were able to utilise As(III) as their sole electron donor. Arsenite oxidation was seen to be catalyzed by a periplasmicarsenite oxidase [34].

I. Uranium: Sulfate-reducing bacteria (SRB) can act directly or indirectly on metals like uranium. One of the studies evaluated in situ &biostimulated activity of SRB present in ground-water influenced soils from a creek bank contaminated with different heavy metals and radionuclides.

They are found to be transformed mainly in anoxic conditions and microbes used for transformation are usually *Desulfobacterales&Desulfovibrionales*, *Syntrophobacteraceae*and*Clostridiales*[35].

J. Petroleum Hydrocarbons: Petroleum hydrocarbons can be classified into four classes namely the saturates, the aromatics, the asphaltenes, and the resins. Degradation of alkylaromatic compounds were seen to be caused by *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas and Rhodococcus.* Microbial transformation of petroleum hydrocarbons in a polluted tropical stream are usually caused by *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenessp.,Acinetobacterlwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium sp.* Majorly the enzymes participating were found to be Cytochrome P450 and alkane hydroxylases which constitute a super family of ubiquitous Heme-thiolateMonooxygenases. These enzymes play an important role in the microbial reduction of oil, chlorinated hydrocarbons, fuel additives and many other such compounds. Cytochrome P450 was isolated from species like *Candida maltose, Candida tropicalis* and *Candida apicola*.

K. 1,2,3-Trichloropropane (TCP): TCP is a major groundwater pollutant and is suspected as a human carcinogen. TCP is a xenobiotic chlorinated compound having a very high chemical stability. The biodegradation includes pathways like reductive dechlorination, monooxygenase-mediated cometabolism, and enzymatic hydrolysis. TCP is usually found to be degraded in anaerobic conditions.

The microbes common for degrading TCP are *Dehalogenimonaslykanthroporepellens* (BL-DC-8 and BL-DC-9) strains and *Methylosinustrichosporium.* [17]

VI. OTHER MAJOR POLLUTANTS

Other major pollutants that are present both in soil as well water are Trichlorobenzene (TCB), Polycyclic aromatic hydrocarbon, p-cresol, 3-chlorobenzoate, 1,2-dibromoethane, Carbon tetra-

chloride, Toxic selenium ions , Hexachlorobenzenes ,Decarbomodiphenyl ether, Phosphates etc.

A. Trichlorobenzene (TCB): The high volatility and slow biodegradation rate of the TCBs were one of the major experimental hindrances which were partially overcome by using a innovative and specially designed incubation and trapping apparatus. Studies revealed that anaerobic condition had negative effect on mineralization while on the other hand increase in temperature had a positive effect. *Pseudomonas Putida*can oxidise chlorinated benzenes to respective chlorocatechols through a mechanism known as dioxygenase mechanism which finally results in ring dechlorination[36]. However if a greater amount of TCB is added to the soil, the degradation rate usually drops. In a study optimum degradation rate was given when 10µg of TCB was added and it resulted in greater mineralization.

B. Polycyclic aromatic hydrocarbon: One of the experimentsdealt with two types of samples- one being a water sample and another being sediments. These samples along with radio-labelled polycyclic aromatic hydrocarbons were incubated. $^{14}CO_2$ and bound ^{14}C were found to be the major transformation products and soluble ^{14}C was the most notable transformant in all water samples. Continuous inputs of PAH resulted in the increased ability of microbes to transform the PAHs[37]. But soon after PAHs were removed, transformation rates still remained elevated.

C. p-cresol: Periphyton and bacterial samples were collected from few field sites and adaptation lag periods were determined for microbial reduction of p-cresol. It was found that ponds have longer adaptation periods as compared to rivers which possess shorter adaptation periods. The commonly used microbes for transformation of p-cresol are *Rivulariaceae, Tolypothrix* and other N_2 fixing cyanobacterias like *Anabaena, Chroococcus and Oscillatoriaspp [38]*.

D. 3-chlorobenzoate (CBA): Two boreal samples and four Mediterranean soil samples having no immediate exposure to pesticides or human disturbances were collected and studied. 3-CBA was mineralized by 96% of the individual soil samples and 610 strains of 3-CBA degraders were found.

E. 1,2-dibromoethane: 1,2- Dibromoethane (DBE) is a carcinogenic pollutant and generally found in soil and groundwater supplies. A study focussed on the aerobic aspect of microbial biodegradation of pollutants. In the study it was found that DBE and 2-bromoethanol were toxic to the DCE (Dichloroethane) reducing bacteria *Xanthobacterautotrophicus* GJ10 and *Ancylobacteraquaticus*[8].

F. Carbon Tetrachloride: Chlorinated compounds are one of the most prevalent pollutants our environment is dealing with. Among the top 45 organic chemicals, Carbon Tetrachloride (CT) is one of the toxic chemical produced by the United States chemical industry with 143,000 tons being produced in 1991. The aspecificdechlorinating ability of unadaptedacetogenic and methanogenic bacteria was assessed by making use of methanogenicgranular sludge from upflow anaerobic granular sludge blanket (USAB) reactors and CT as a model compound. The sludge was enriched with various acetogenic and methanogenic bacteria. Main end products of CT degradation were CO_2 and Cl^- and the yields of theseendproducts were 44 and 68% respectively of the initial amounts of [^{14}C] CT and CT-Cl. Both living and autoclaved sludges could degrade choloroform however only living sludge could degrade dichloromethane and methyl chlorides. Results revealed that reductive dehalogenation was better mediated by living sludge than by autoclaved sludge. It was observed that Granular Sludge thus has an outstanding potential for gratituitousdechlorination of CT to safer end products [39].

G. Toxic Selenium ions: One of the research focussed on biosynthesis of selenium (Se0) nanospheres by *Bacillus cereus* CM100B. It transformed the toxic (SeO_3^{2-}) anions into reduced elemental Selenium (Se^o). Different sodium selenite concentrations were added to see if the strains were able to tolerate high levels of selenite ion toxicity. The mechanism involved a membrane –associated reductase enzyme(s) that was able to reduce selenite
(SeO_3^{2-}) to (Se^o) through electron shuffle enzymatic metal reduction process. This method seems to operate best under aerobic conditions [40].

H. Hexachlorobenzenes (HCB): Organohalides e.g. HCB are recalcitrant to aerobic microbial reduction. HCB is one of the constituent of pesticides. It is quite toxic and carcinogenic. It is a resultant of volcano emissions which is reduced in an anaerobic process. This study focussed on the hexachlorobenzene (HCB) dechlorination ability of Dehalococcoides spp. in the process. Three strains of bacteria capable of degrading HCB via reductive dechlorination that have been isolated till noware *Dehalococcoides sp*. Strain CBDB1, *dehalococcoidesethenogens* strain 195 and *Dehalobiumchlorocoercia* DF-1 [15].

VII. BIOREACTORS USED IN MICROBIAL TRANSFORMATION

Reactors and bioreactors are one of the most important components of technological production system. The most crucial part is to select a proper reactor and to set the optimal parameters. Although

reactor engineering gives a suitable solution for these problems, narrowing down the work parameters for enzymatic reaction (especially for microbiological transformation) is still a severe problem.

A bioreactor is a system which is used to carry out a biological conversion. The bioreactors referred here are specified for various water and soil pollutants. These bioreactors consist of mechanical vessels in which

A. organisms are cultivated in a controlled manner

B. Materials are converted or transformed via specific reactions.

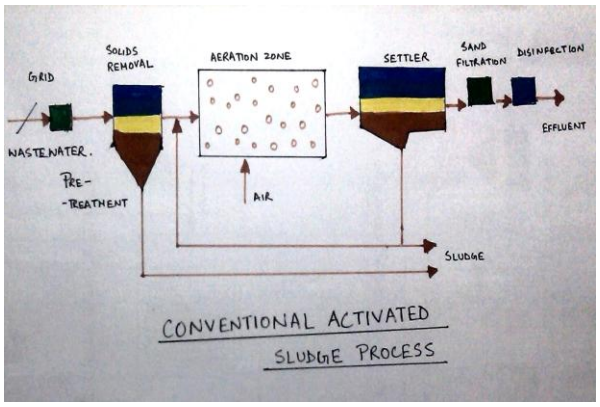

Fig.2. Flow diagram of conventional bioreactor**.**

VIII. MEMBRANE BIOREACTOR FOR WASTE WATER TREATMENT

The Membrane bioreactor (MBR) uses combination of both processes- microfiltration and ultra filtration. A suspended bioreactor used for growth is used mainly for industrial as well as municipal wastewater treatment which has got a plant size which is equivalent to that of 80,000 populations.

Membrane bioreactor processes can effectively produce effluent of very high quality, when used along with domestic wastewater and can be effectively discharged to surface, brackish or coastal waterways or can be used in urban irrigation[42]. The one advantage that MBRs have over other conventional processes consist of easy retrofit, small footprint as well as the upgrade of other old wastewater treatment plans. MBRs have now become a very established process for treating the wastewaters as the recent innovations in technical and also significant reduction in cost of membranes have enabled them to work this way. As a result of which MBR process is now an attractive option for treating wastewaters and is also used for reusing municipal and industrial wastewaters as can be seen from their consistently rising capacity and numbers. The recent market for MBR is estimated to be valued at around US $216 million in the year 2006 and then rose to US $363 million by the year 2010. The reduction of footprints of activated sludge and sewage treatment system has been carried out by MBRs by removing a part of the liquid component from the mixed liquor. This results in a waste product which is concentrated and then it is treated by utilizing the activated sludge process. The configuration of the submerged system relies on the coarse bubble aeration to result in the mixing and to reduce fouling. In the submerged configurations, one of the major parameters is aeration in terms of both biological as well as hydraulic. Aeration scours the surface of the membrane, provides the biomass with some oxygen and also maintains all the solids in suspension, thus resulting is a better cell synthesis and biodegradability. There are two different types of MBR configurations- internal/submerged where the membranes have to be immersed in and is integral to biological reactor; and external/side stream, in which the membranes are completely separate process which requires a pumping step which is immediate.

A.INTERNAL/SUBMERGED

In the main bioreactor vessel or in a tank attached separately, the filtration element is attached. The membranes can be of different forms and can be tubular or flat sheet or a combination of both. These membranes can bring about an online backwash system that significantly reduces fouling on the surface of the membranes by pumping the permeate that is formed back through the membrane. In many setups, the membranes in separate tank can be retrieved to undergo cleaning using membrane soaks, however the biomass that is present must continuously be pumped into the main reactor to put a check on the concentration of MLSS (Mixed Liquor Suspended Solids), which must not exceed a given amount. To reduce fouling, air scour is provided by additional aeration.

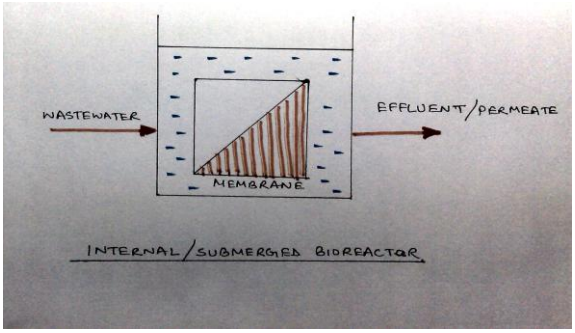

Fig.3. The diagram shows how wastewater is treated in an internal/submerged bioreactor.

B. EXTERNAL/SIDESTREAM

In a plant reactor, to the external side of the reactor, the elements of filtration are installed. The biomass that is present is either directly pumped via a particular number of membrane modules arranged in series and then back to the bioreactor, or to a bank of membrane modules, the biomass is pumped into it, from where a secondary pump moves about the biomass through the membrane modules, arranged in series. By using an installed pump, pipe work and cleaning tank, the soaking and cleaning of the membranes can be carried out.

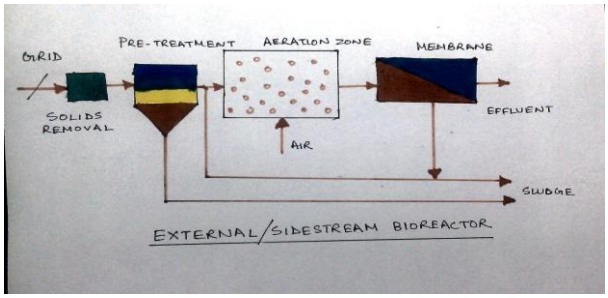

Fig.4. The flow diagram depicts the working of an external/sidestream bioreactor.

IX. MAJOR LIMITATIONS AND CONCERNS IN MBR

In MBR, the major concerns and limitations are as follows:

A. Fouling and its control:

With filtration time, the performance of MBR filtration decreases considerably. This happens because the particulate and soluble materials get deposited into and onto the membrane that results in the interactions between the membrane and the sludge components which have been activated. The major process limitation and drawback that has been looked into from the beginning of the MBR's formed early and it still remains as one of the most challenging issues faced during the course of development of the MBRs. In recent reviews regarding the applications of membrane in bioreactors, it has been found out that the most serious problem affecting the performance of the system is membrane fouling. Hydraulic resistance increases significantly due to fouling which is manifested as a decline in permeate flux or as an increase in transmembrane pressure (TMP) when the operation of the process occurs under constant-flux or constant-TMP conditions respectively. In some of the systems, by increasing the TMP, the flux is maintained and the energy that is required to obtain filtration increases significantly. The operating costs are increased considerably as a result of production downtime and cleaning agents which are required frequently for membrane cleaning. Frequent replacements of membranes are also expected, which further increases the operation costs. Because of the interaction between the components of the sludge (including biological flocs formed a vast range of dead or living microorganisms along with other colloidal and soluble compounds) that has been activated and the material of the membrane, membrane fouling occurs. The biomass that is suspended does not have a fixed composition and varies considerably with the composition of the feed water and the conditions that is employed in MBR. Thus, even though various investigations regarding the fouling of the membrane have been carried out, the wide range of feed water matrices and operating conditions employed, the various analytical methods employed and the restricted information reported in most of the published studies on the composition of the suspended biomass, has made it difficult to come out with any behaviour that is generic to this membrane fouling occurring specifically in MBRs.

There are various strategies resulting in anti-fouling that can be applied to MBRs applications and has been mentioned below:

1) Intermittent Permeation: Over here, the filtration of the membrane is stopped regularly at given time intervals ie. Around two minutes before it is resumed again. The particles diffuse right back to the reactor, where the particles were deposited on the surface of the membrane initially.

2) Membrane Backwashing: The internal and external foulants are dislodged as the water from thepermeate is pumped right back to the membrane and to the feed channel via the pores.

3) Air Backwashing: Over here, the building of the pressurized air occurs on the side of the membrane where permeate is present and this occurs within a short interval of time. Therefore, the membrane modules have to be in a vessel which is pressurized and is coupled to a vent system. Normally, air do not flow through the membrane, however if it actually did, the membrane would become dry upon its exposure to the air and another step would be required to wet the membrane again, by pressurizing the membrane on its feed side.

4) Chemically Enhanced Backwash, that occurs daily.

5) Cleaning done for maintenance by using higher concentration of chemical and this occurs weekly.

6) Intensive chemical cleaning that occurs once or twice in a year.

B. Nutrient Removal Due To Eutrophication

In the areas that are susceptible to eutrophication, removal of nutrient is a major concern. The most applied technology used for the removal of Nitrogen from the municipal waste water is both nitrification and occurring simultaneously. Apart from phosphorous precipitation, enhanced biological phosphorous removal (EBPR) can also be used which a supplementary anaerobic process needs step. Some of the characteristics of MBR technology makes EBPR coupled with post-denitrification a good alternative that successfully achieve very low concentrations of nutrient effluent.

X. SLURRY BIOREACTORS

Only in the presence of a pre-treated feed stock that a slurry bioreactor functions properly, therefore a dewatering operation and washing-separation operations are integrated with the bioreactor. In the given figure, a general set up of an integrated slurry bioprocess is present. Initially, using a vibrating wet screen, the feed stock is screened for the removal of the debris that is present are mainly of size of 2-6mm. Thereafter, by using one or many techniques for separation namely, floatation cells, hydroclones, sieves, upflow column, Humphrey spirals and jigs, the sand fractions are removed successfully. The flow of the slurry inside the cyclone is divided into fine particles at the top ie particle size <63 µm into a fraction of sand particles of particle size > 63 µm. The top fraction of the cyclone that contains the contaminated fine particles then fed into the bioreactors. A dewatered product which contains the fine particles and process water flow results from the final operation.

A. BATCH OPERATION

In a slurry bioreactor, the major components are baffles, a sparger present at the bottom and a mechanical stirrer. A slurry bioreactor is also often called as a standard continuously stirred tank reactor (CSTR). The given phase has a three phase component of water, air and contaminated solids is maintained. Experiments regarding batch degradation have been frequently carried out in stirred slurry bioreactors which have been aerated.

1) When the biomass is batch processed which grows on a complex substrate, that has to adapt to different components continuously as the parts which are easy gets degraded first. This setup inevitably leads to a lot of difficulties for the population of microbes that are present. The contaminants that are present are insufficient for the population that is present to feed on.

2) The desorption kinetics of the contaminants are of such order that when the concentrations are lower, the “driving force” gets limited for the contaminants that are absorbed and which waits to be released from the solid particles that are present. The microbial degradation proceeds only when the contaminant gets completely dissolved in the phase containing water and this kind of kinetics does not let easy degradation at concentrations lower than what should be necessary.

3) During the batch process, the side products that are inhibiting and are formed from the microbial degradation may be released increasingly into the medium, also, the solid conditions may physically change which might lead to microbial circumstances that are unfavourable. For example, due to a humification process, a pH drop can join in the breakdown of the contaminant.

Nonhalogenetaed semi volatile organic compounds (SVOCs), petroleum hydrocarbons, volatile organic compounds (VOCs) and explosive compounds which are present in soil are primary treated by slurry bioreactors. Microorganisms that are specially adapted and co-metabolite containing slurry phase bioreactors are used in treating both halogenated SVOCs and VOCs, polychlorinated biphenyls (PCBs) and pesticides present in excavated dredged sediments and soils.

B. MAJOR LIMITATIONS AND CONCERNS IN SLURRY BIOREACTORS

The particulate and dust emissions should be brought under control as the excavation of the contaminated media is required. Any contaminants which are free-phase should be removed before mixing the soil with the slurry. If very high concentrations of contaminants are present, it may get toxic to the microorganisms. It might get very expensive to dry the soil after the treatment. Residual contaminants should also be carefully monitored for. In specific site/soil condition, the biodegradation of contaminants which are specific depends on various factors such as soil chemistry, soil type, the mix of temperature and contaminants. It is important to characterize the soil, contaminations and the site and also to evaluate the potential for biodegradation of the contaminants to determine the appropriate remedy for biodegradation. A treatability study which is preliminary must be conducted. A method which is acceptable for disposing off wastewater and is non-recycled is required. The biodegradation rates can be decreased by using low ambient temperature. The can also be toxic to microorganisms.

XI. CONCLUSIONS

Through many researches that were conducted on the rates of microbial transformation of pollutants we understood that there are many factors that play a very important role in determining the rates.

Some of the crucial factors are pH, temperature and the microenvironment (including consortia). It was seen that there is no particular fashion of selecting only aerobic and anaerobic methods. Both these are used depending on the microorganisms and the substrates. We also came to know about different pathways and enzymes involved in those metabolic pathways. All this knowledge can be processed further and new genetically recombinant strains can be generated, which might help in degrading the recalcitrants which are yet not biodegradable.

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