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Research Article

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Environmental Bioremediation: A Low Cost Nature's Natural Biotechnology for Environmental Clean-up

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Abstract

Setting up of new industries or expansion of existing industrial establishments resulted in the disposal of industrial effluents, which discharge untreated effluents causing air, water, soil and solid waste pollution. Bioremediation is an ecologically sound and state-of-the-art technique that employs natural biological processes employing microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Compared with other technologies, such as thermal desorption and incineration, thermally enhanced recovery, chemical treatment, and *in situ* soil flushing (which may require further management of the flushing water), bioremediation may enjoy a cost advantage. Not all contaminants, however, are easily treated by bioremediation using microorganisms. While bioremediation can't degrade inorganic contaminants, can be used to change the valence state of inorganic and cause adsorption, immobilization onto soil particulates, precipitation, uptake, accumulation, and concentration of inorganic in micro or microorganisms. This manuscript delineates the general processes of bioremediation within the soil environment, factors of bioremediation strategies, genetic engineering approaches, monitoring bioremediation, and further, the *pros* & *cons* of the technique, limitations and potential of both *ex situ* and *in situ* bioremediation as viable alternatives to conventional remediation are explained and addressed.

Keywords: Bioremediation; Hazardous substances; Effluent treatment plant; *in situ; ex situ*

Introduction

Even though we can travel to the Moon, send robots to Mars, make super computers and clone organisms we still have difficulties to clean the water we use. In many parts of the world the availability of water is a crucial issue, and even more so, clean water. Environmental pollution is the most horrible ecological crisis that man is facing today. Pollution is a global threat to the environment and it becomes a scare word of today's world. The rapid growth of human populations fuelled by technological developments in health and agriculture has led to a rapid increase in environmental pollution. The unprecedented population increase and industrial development during the 20^{th} century has not only increased conventional solid and liquid waste pollutants to critical levels but also produced a range of previously unknown pollution problems for which society was unprepared. The growth of the world population, the development of various industries, and the use of fertilizers and pesticides in modern agriculture has overloaded not only the water resources but also the atmosphere and the soil with pollutants [1-5]. In the last few decades the handling of wastewater appeared to be one of the most important. The degradation of the environment due to the discharge of polluting wastewater from industrial sources is a real problem in several countries. This situation is even worse in developing countries like India where little or no treatment is carried out before the discharge [6]. In spite of the many steps taken to maintain and improve the quality of surface and groundwater, the quantities of wastewater generated by these industries continue to increase and municipalities and industries are confronted with an urgent need to develop safe and feasible alternative practices for wastewater management Bioremediation is a pollution-control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms.

Xenobiotic compounds are not naturally available and hence the locally occurring microorganisms cannot readily degrade them. Hazardous materials may render harm to humans, livestock, wildlife, crops or native plants through handling, ingestion, application to land or other distributions of the contaminated materials into the environment. The textile industry leaves about 50% of the textile Azo dyes in Free State to be discharged in the factory effluent and eventually to the surrounding environment. Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paper printing [7]. The reactive Azo dyes containing effluents cause serious environmental pollution. Therefore, industrial effluents containing Azo dyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents [7]. The planet 'Earth' is endowed with rich wealth of natural resources such as forests, wildlife, land, soil, air, water, wind, plants and animals. The race begins when humans started living a stable life rather than a nomadic life. Since the advent of civilization the use and overuse, and now the misuse has led to depletion of various natural resources to an extent that today half of our natural wealth are either depleted or at the edge of depletion [8]. In early times, it was believed that our land and its resources are in abundance and will remain available for decades. However, today existing state of our resources shows carelessness and negligence in using them. The industrial and anthropogenic activities had also led to

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the contamination of agricultural lands resulting in loss of biodiversity. The biodiversity of plant and animal species play important role in the development of healthy and productive ecosystem and, thus contribute to large number of economic benefits to man and environment. Unfortunately, rapidly growing population and increased human activity has threatened many of these species.

The natural processes such as crude oil formation, soil formation, waste disposal, nitrogen fixation, biological pest control, pharmaceutical production, dispersal of fruits and pollination are all accomplished by the enormous biodiversity available worldwide [9]. The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective in reducing levels of a range of contaminants, but have several drawbacks, principally their technologies complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants that may increase the exposure to contaminants for both the workers at the site and nearby residents. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable. However, as the range of contaminants on which it is effective is limited, the same time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. Because bioremediation seems to be a good alternative to conventional clean-up technologies research in this field, especially in the US is rapidly increasing.

Three primary ingredients for bioremediation are: 1) presence of a contaminant, 2) an electron acceptor, and 3) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved. Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic chemicals such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such contaminants into less toxic forms. Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are nutrient availability, moisture content, pH, and temperature of the soil matrix. Inorganic nutrients including, but not limited to, N & P are necessary for microbial activity and cell growth. It has been shown that treating petroleum-contaminated soil with nitrogen can increase cell growth rate, decrease the microbial lag phase, help to maintain microbial populations at high activity levels, and increase the rate of hydrocarbon degradation [10].

All soil microorganisms require moisture for cell growth and function. Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process. Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6-8. Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms. Generally, speed of enzymatic reactions in the cell ~ doubles for each 10°C rise in temperature. There is an upper limit to the temperature that microorganisms can withstand. Most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbons, are mesophiles which have an optimum temperature ranging from 25°C to 45°C. Thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60°C. This finding suggested an intrinsic potential for natural attenuation in cool soils through thermally enhanced bioremediation techniques [11]. Contaminants can adsorb to soil particles, 1 rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type. Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants. Additional research findings of the existence of thermophilic bacteria in cool soil also suggest that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants. It is suggested that contaminants adsorbed to soil particles are mobilized and their solubility increased by high temperatures.

If the challenges of bioremediation, particularly of *in situ* techniques, can be overcome, bioremediation has potential to provide a low cost, non-intrusive, natural method to render toxic substances in soil less harmful or harmless over time. On a broader scope, much research has been and continues to be developed enhance understanding of the essence of microbial behaviour as microbes interact with various toxic contaminants. Additional research continues to evaluate conditions for successful introduction of exogenic and genetically engineered microbes into a contaminated environment, and how to translate success in the laboratory to success in the field [12]. Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. Large amounts of toxic waste have been dispersed in thousands of contaminated sites spread across our nation. Thus every one of us is being exposed to contamination from past and present industrial practices, emissions in natural resources (air, water

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and soil) even in the most remote regions. The risk to human and environmental health is rising and there is evidence that this cocktail of pollutants is a contributor to the global epidemic of cancer, and other degenerative diseases (Figure 1).

These pollutants belong to two main classes: inorganic and organic. The challenge is to develop innovative and cost-effective solutions to decontaminate polluted environments, to make them safe for human habitation and consumption, and to protect the functioning of the ecosystems which support life. Much progress has been made in developed countries like UK, USA, Canada, Australia, Japan and European countries. However, in India there is an urgent need to evaluate the exciting developments coming out of various laboratories. Bioremediation is the use of biological interventions of biodiversity for mitigation (and wherever possible, complete elimination) of the noxious effects caused by environmental pollutants in a given site. It operates through the principles of biogeochemical cycling (Figures 2 and 3). If the process occurs in the same place affected by pollution then it is called *in-situ* bioremediation. In contrast, deliberate relocation of the contaminated material (soil and water) to a different place to accelerate bio catalysis is referred to as *ex-situ* bioremediation. Bioremediation has been successfully applied for cleanup of soil, surface water, groundwater, sediments and ecosystem restoration. It has been unequivocally demonstrated that a number of xenobiotics including nitro-glycerine (explosive) can be cleaned up through bioremediation. Bioremediation is generally considered to include natural attenuation (little or no human action), bio-stimulation or bio-augmentation, the deliberate addition of natural or engineered micro-organisms to accelerate the desired catalytic capabilities Thus bioremediation, phyto remediation and rhizo remediation contribute significantly to the fate of hazardous waste and can be used to remove these unwanted compounds from the biosphere (Figure 4).

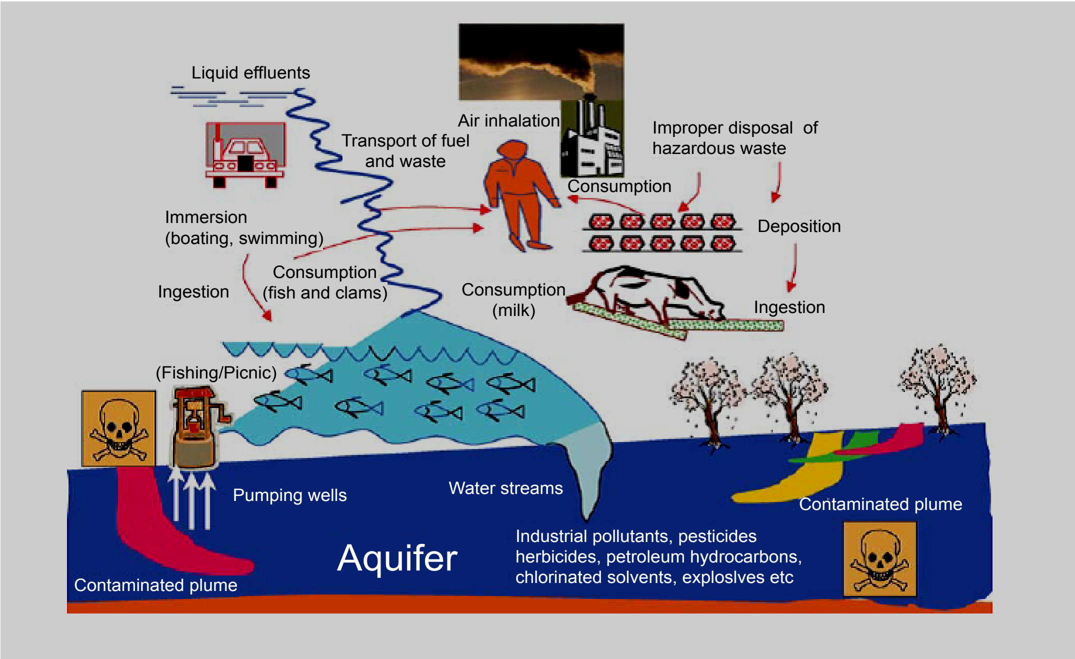

Figure 1: Fate and transport of organic/inorganic contaminants/pollutants and their harmful effects.

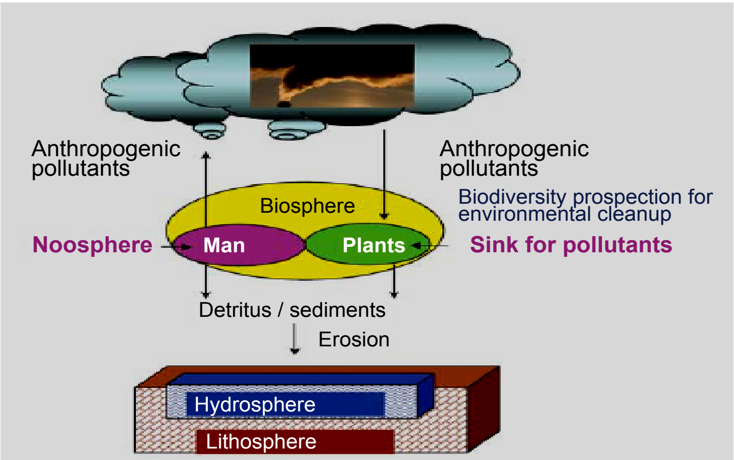

Figure 2: Biogeochemical cycle and its connection to bioremediation.

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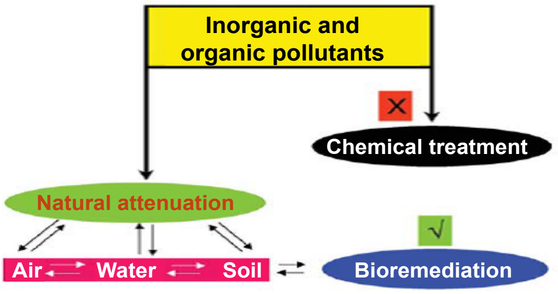

Figure 3: Natural attenuation and bioremediation are widely accepted for environmental cleanup.

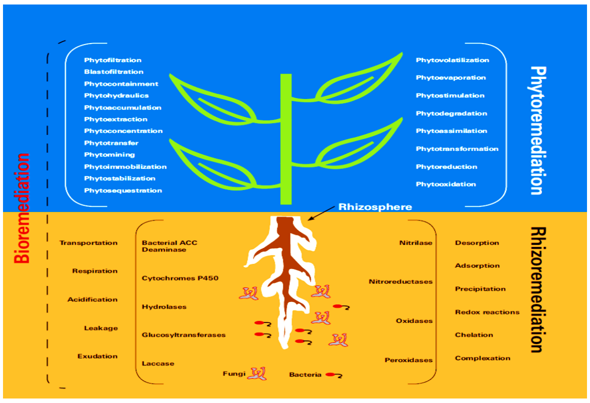

Figure 4: A State of the Art of Bioremediation Strategy.

Bio remedial approach

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". Technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere). Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth of the pollution-eating microbes within the medium (bio stimulation). Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals, and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes [12-15].

Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as Cd & Pb are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil. Bone char has been shown to bioremediate small amounts of Cd, Cu & Zn. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use [16]. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes [17].

By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated sites. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have process known as bio augmentation [18]. For bio augmentation to be effective, microorganisms must enzymatically attack the pollutant and convert them to harmless products. A bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Like other technologies, bioremediation has its limitations [19].

Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a remediation; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more accepted than other technologies. Most remediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules [20].

Inimitability for remediation

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) [21].

Microbial decolouration mechanisms

Many processes were employed to remove dye molecules from industry effluents and the treatment methods can be divided into the following categories:

Physical methods: Physical methods such as adsorption [22,23], ion exchange [24-28] and membrane filtration [25] were employed in the removal of dyes. The main disadvantages of these physical methods were they simply transfer the dye molecules to another phase rather than destroying them and they were effective only when the effluent volume is small [26,27].

By adsorption: Adsorption is the transfer of solute dye molecule at the interface between two immiscible phases in contact with one another. The removal of colour from dye industrial effluents by the adsorption process using granular activated carbon has emerged as a practical and economical approach [29].

By Ion Exchange: Removal of Anions and Cations from dye industry effluent can be carried out by Ion exchange method by passing the waste water through the beds of ion exchange resins where some undesirable cations or anions of waste water get exchanged for sodium or hydrogen ions of the resin. Greluk and Hubicki recommended the adsorption/ion exchange as an alternative method for the removal of reactive dyes. The applicability of ion exchange resin containing acrylic matrix for removing other classes of dyes were well documented by Bayramoglu et al. [30], Dulman et al. [31], Wawrzkiewicz and Hubicki [32] and Barsanescu et al. [33]. Thus Acrylic anion exchangers is more advantage than styrenics by exhibiting high efficiency of anion exchange capacities and polluting less.

By membrane filtration

Reverse osmosis (RO) and electro dialysis are the important examples of membrane filtration technology. The contribution of reverse osmosis in removing this high salt concentration is of great. This RO reject can be reused again in the process. For reactive dyeing on cotton, the presence of electrolytes in the waste water causes an increase in the hydrolyzed dye affinity making it difficult to extract. The total dissolved solids from waste water were removed by reverse osmosis. Though it is suitable for removing ions and larger species from dye bath effluents with high efficiency, it possesses some disadvantages like clogging of the membrane by dyes after long usage and high capital cost. In electro dialysis, the dissolved salts (ionic in nature) can also be removed by impressing an electrical potential across the water, resulting in the migration of cations and anions to respective electrodes via anionic and cationic permeable membranes. To avoid membrane fouling it is essential that turbidity, suspended solids, colloids and trace organics are to be removed prior to electro dialysis.

Chemical methods

Chemical methods such as chemical oxidation [34], electrochemical degradation [35], and ozonation [36] were employed in dye removal effectively. A variety of oxidizing agents were used to decolorize wastes by oxidation techniques effectively. Among that sodium hypochlorite decolorizes dye bath efficiently. Even though it is a low cost technique, it forms absorbable toxic organic halides. Ozone on decomposition generates oxygen and free radicals. The main disadvantage of this technique is that it requires an effective sludge producing pretreatment.

Advanced Oxidation Process (AOP)

Philippe et al. [37] Slokar and Le Marechal [38] were reported that the conventional water treatment technologies such as solvent extraction, activated carbon adsorption and chemical treatment process such as oxidation by ozone (O_3) often produce hazardous by products and generate large amount of solid wastes, which require costly disposal or regeneration method. Due to these reasons, Mahadwad et al. [39] considerable attention had been focused on complete oxidation of organic compounds to harmless products such as CO_2 and H_2O by the AOP.

Electrochemical method

The requirement of chemicals and the temperature to carry the electro chemical reaction is less than those of other equivalent non-electrochemical treatment. It can also prevent the production of unwanted side products. But, if suspended or colloidal solids were high in concentration in the waste water, they slow down the electrochemical reaction. Therefore, those materials need to be sufficiently removed before electrochemical oxidation. Ceron et al. [40] reported that many of the commercially used dyes are resistant to biological and physico-chemical methods [41]. Consequently, Yang et al. [42] reported a new result for the color removal of dye from wastewater by applying electro generated hypochlorite ions and (Ru + Pt) Ox binary electrodes. Even if the removal of dyes from wastewater in an economic way by using electrochemical method, the low-cost electrode production remains a major concern. Chatterjee et al. [43] also reported that due to its effective electron donating capacity, ZVI particles had been studied for the treatment of wastewater contaminated with chlorinated compounds, nitro aromatic compounds, nitrates, heavy metals, organochlorine pesticides, and dyes. Saxe et al. [44] reported that ZVI particles convert azo dye into some products that were more susceptible to biological degradation process.

Biological methods

Bioaccumulation and biosorption are the two main technologies in biological process for of dye bearing industrial effluents. They possess good potential to replace conventional methods for the treatment of dyes industry effluents [45]. Biological process can be carried out in situ at the contaminated site, these are usually environmentally benign i.e., no secondary pollution and they were cost effective. These are the principle advantages of biological technologies for the treatment of dye industry effluents. Hence in recent years, research attention has been focused greatly on biological methods for the treatment of effluents [46]. The disadvantage of this degradation process is that it suffers from low degradation efficiency or even no degradation for some dyes [47] and practical difficulty in continuous process. Vijayaraghavan and Yun [46] clearly demonstrated the difference between bioaccumulation and biosorption in their review. Bioaccumulation is defined as the phenomenon of uptake of toxicants by living cells; whereas, biosorption can be defined as the passive uptake of toxicants by dead or inactive biological materials. The important advantage of biosorption than bioaccumulation process is the use of living organisms is not advisable for the continuous treatment of highly toxic effluents. This problem can be overcome by the use of dead biomass, which is flexible to environmental conditions and toxicant concentrations. Wang et al. [47] reported that adsorption rather than degradation plays a major role during the decolourization process by fungi and algae.

Microbial Population for Bioremediation Processes

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards [48-50]. We can subdivide these microorganisms in Table 1.

Class of contaminants	Specific examples	Aerobic	Anaerobic	More potential sources
Chlorinated solvents	Tri chloroethylene, Per chloroethylene		+	Dry cleaners
Polychlorinated bi phenyls	4-chlorobiphenyl, 4,4 di chlorobiphenyl		+	Electrical manufacturing PowerStation
Chlorinated phenol BTEX	Pentachlorophenol,bnzene,Toulene,Ethyl benzene,Xylene	+	+ +	Timber treatment Landfills, Oil production and storage gas work sites, Airport paint manufacture, Port facilities, Railway yards, Chemical manufacture
Poly aromatic hydrocarbons Pesticides	Naphthalene, Anthracene Benzopyrene Atrazine carbaryl Carbofuran Diazine Glycophosphate ParathionPropham2,4D	+ +	+	Oil production and storage gas work sites, Coke plant engine works landfill, Agriculture timber treatment plants, Pesticides manufacture, Recreational areas landfills

Table 1: Some contaminants potentially suitable for bio remediation.

Organism	Dyes	Activity	Decolouration	Comments	References
Clostridium perfringens ATCC 3626	Amaranth Methyl Orange Orange II Tartrazine	μmol/ml/h 0.74 0.62 0.70 0.67	- - - -	Dye concentration of 0.033 mM	[54]
Pseudomonas GM3	Acid Violet 7 Reactive Blue 2 Acid Green 27 Acid red 183 Indigo Carmine	- - - - -	97.4 18.3 75.6 20.1 69.0	After 72h of incubation. Dye concentration of 100 mg/l	[55]
Enterococcus faecalis	Methyl Red Orange II OrangeG Amaranth	AUx10^{-2}/mg protein 1.81 1.39 1.20 1.37	99.84 95.1 64.1 99.5	After 20 h of incubation. Dye concentration of 0.2 mM	[56]
Eubacterium biforme	Tartrazine Sunset Yellow Methyl Orange Orange II Amaranth Allura Red 40	- - - - - -	4.0 22.0 79.0 81.0 19.0 11.0	After 150 minutes of incubation. Dye concentration of 2 mM	[57]

Table 2: Facultative and strictly anaerobic bacterial cultures, which are able to decolorize Azo dyes under anaerobic conditions.

Microbiological Aspects of the Reductive Decolorization of Azo Dyes

Pure cultures

As reflected in Table 2, there are extensive reports for use of pure cultures, either whole cells or specific enzymes, for a better insight of the anaerobic azo dye reduction mechanisms, which are not fully understood yet [51,52]. The understanding of azo dye reduction mechanisms is important not only for a biotechnological approach for decolourisation, but also for a medical approach to have an insight into how the intestinal microflora metabolites ingested azo dyes [53]. Azo dyes are converted into aromatic amines in the presence of microflora and the anaerobic condition in the human intestine. Aromatic amines are more mutagenic and carcinogenic than their precursor, azo dyes [54]. Therefore, a lot of effort has been made in the production of compounds, which are resistant to these reductive transformations. Another approach has been looked into use of azo polymers that would be insoluble in the upper gastrointes-reduction.

Granular sludge

Even though anaerobic azo dye reduction could be readily achieved with different microorganisms, there is no strain reported so far that is able to decolourise a broad range of azo dyes. Therefore, the use of a specific strain or enzymes for reductive decolourisation does not make much sense in treating textile wastewater, which containing many kinds of dyes. The use of mixed cultures, such as anaerobic granular sludge, which is composed of stable microbial pellets with a high activity, is probably a more logical alternative. However, a little is known about the microbiological aspects of the reductive decolourisation of azo dyes with anaerobic consortia, commonly found in wastewater treatment plants, although the applicability of the cost-effective high-rate anaerobic reactors for azo dye reduction has been well demonstrated [52-60]. As previously explained, the reductive decolourisation of azo dyes by using methanogenic anaerobic granular sludge is likely to be controlled by a co-metabolic reaction in the presence of different electron donors, in which the azo dye is the terminal electron acceptor of the reduced co-factors [60].

Non-biological colour removal

While advanced oxidation processes (AOPs) have been studied extensively both for recalcitrant wastewater in general and dye wastewater in particular, their commercialization has yet not been realized because of certain barriers [61,62]. These processes are costly and complex also at the present level of their development [63]. Additional impediment exists in the treatment of dye wastewater with higher concentration of dyes, as AOPs are only effective for wastewater with very low concentrations of organic dyes. Thus, significant dilution is necessary for the wastewater treatment. For the AOPs, the basic reaction mechanism is the generation of free radicals and their subsequent attack on the organic pollutant species. The cost/energy efficiency, however, will be dependent on the operating conditions and the type of the wastewater.

Factors that Control Microbial Dye Decolouration

Microorganisms are sensitive to the presence of chemical substances, such as dyes, high salinity, variations in pH and high content of organic compounds [64-66]. For bioremediation processes, the most useful microorganisms are those isolated from textile industry-contaminated environments, including soil, effluents and sludge from wastewater treatment plants, because they are adapted to grow in extreme conditions [67-69]. The bio decolouration process is dependent on the following factors: the azo dye structure, carbon and nitrogen sources, salinity, pH, temperature, dye concentration and the presence or absence of oxygen.

Effects of the azo dye structure

It has been observed that the enzymatic degradation of the dye is highly influenced by its structure [70-74]. Recent studies have revealed that the enzymatic activity is induced by the presence of dyes in such a way that this activity is significantly higher at the end of the decolouration process. For example, Reactive Black 5 induces MnP activity in *Debaryomyces polymorphus* [70] and *Trichosporon akiyoshidainum* and Lac activity in *Trametes versicolor* [71]; azoreductase activity is improved in *Chlorella vulgaris* by the addition of G-Red, and in *Scenedesmus bijugatus* by Tartrazine and Ponceau [72]; Reactive Blue 221 induces Tyr in *T. akiyoshidainum* [73] Lac activity is enhanced in *Pleurotus sajorcaju* in the presence of Acid Blue 80, Acid Green 28 and Reactive Red 198 [74] and in *Galactomyces geotrichum* by a mixture of Remazol Red, Golden Yellow HER, Rubine GFL, Scarlet RR, Methyl Red, Brown 3 REL and Brilliant Blue [75]. Throughout the process of azo dye biotransformation, different types of enzymes, both oxidises and reductases, can be involved. During the decolouration of Reactive Orange 16 by *Irpex lacteus,* significant amounts of Lac and MnP were identified; three metabolites but not polymerisation products were detected by LC-MS analyses. Even other fungal Lac enzymes can catalyse polymerisation. The use of microorganisms that excrete various oxidases and reductases can completely degrade azo dyes and detoxify contaminated water from the textile industry.

Influence of carbon and nitrogen sources in the decolouration process

Carbon and nitrogen sources have an important influence on the extent of decolouration using microorganisms. Carbon sources have two purposes: as sources of carbon and energy for the growth and survival of the microorganisms and as electron donors, which are necessary for the breakage of the azo bond [76]. Carbon sources are accepted differently by different microorganisms and have an important effect on the extent of decolouration. In several cases, the microbial decolouration of azo dyes or textile effluents is increased in the presence of glucose. Starch is another common source of carbon that is frequently used as an additive in the textile finishing process. Therefore, the use of microorganisms that can use starch as a co substrate would be beneficial for the treatment of wastewater from the textile industry [77]. In addition to the type of the carbon source, it is important to consider the amount of the source because it must be sufficient to meet microbial growth requirements and achieve decolouration. However, high carbon concentrations lead to low decolouration because the microorganisms utilise the carbon source preferentially to the dye [78]. An interesting goal is for microorganisms to use the dye as a carbon source or even as the sole source of carbon and nitrogen. This goal can be achieved in some cases if the microorganisms are acclimatised by successively increasing the amount of dye and diminishing the carbon source until they can survive with the azo dye alone. Nitrogen sources are also important for microbial decolouration. The metabolism of organic nitrogen sources is considered essential for the regeneration of NADH [79]. Different nitrogen sources have been studied to improve the decolouration rate.

Influence of salinity, dye concentration, pH, temperature and oxygen in the decolouration process

The operation conditions affect the efficiency of microorganisms to decolourate azo dyes, such as the presence of salts, concentration of the dyes, pH, temperature and oxygen. Generally, a sodium concentration above 3000 ppm causes moderate inhibition of most bacterial activities [80]; thus, azo dye removal efficiencies under saline conditions decrease. However, there are examples of halo tolerant microorganisms that are able to decolourate azo dyes in the presence of salts [81]. The dye concentration has effects on microbial azo dye decolouration. For example, *Lysinibacillus sp*. Effectively decolourates 100% of Metanil Yellow at 200 ppm but was only able to decolourate 62% of Metanil Yellow at 1000 ppm [80]. *Sphingomonas paucimobilis* decoloured completely Methyl Red at 750 ppm, whereas only 38% of a 1000 ppm dye solution was decoloured [82]. Adsorption and enzymatic activity is dependent on the pH. As the extent of decolouration is influenced by the pH of the media, pH also affects the colour of the solution and the solubility of the dye. *Candida tropicalis* adsorbs 45% of Basic Violet 3 at pH 3, 85% at pH 4 and 33% at pH 9 [83]. *Micrococcus sp.* decolourates 65% of 300 ppm of Orange MR at pH 4, 80% at pH 6, and 40% at pH 10 [84]. Congo Red is decoloured with the bacteria VT-II at 13, 56, 70 and 7% at pH 2, 5, 7 and 10, respectively [85]. Temperature affects microbial growth and enzyme production and, consequently, the percentage of decolouration. For example, *Micrococcus sp*. decolours 60% of 300 ppm of Orange MR at 30°C, 80% at 35°C and 42% at 45°C [84]. *Pseudomonas aeruginosa* degrades 97% of 50 ppm of Remazol Red at 40°C, 72% at 10°C and 82% at 30°C [86]. The presence of oxygen can either favour or inhibit the microbial degradation of azo dyes. Shaking increases mass and oxygen transfer between cells and the medium, and enzyme activity can depend on the presence of oxygen if the mechanism is aerobic. The time to decolour Methyl Red using a *Micrococcus* strain was reduced from 24 to 6 h under a supply of oxygen [87]. *Shewanella oneidensis* shows higher dye decolouration under static conditions (97%) than in aeration (8%), although cell growth is comparatively faster under shaking conditions [88]. Orange II decolouration with *A. niger* improves with shaking (84%) compared with static growth (61%) [89].

Bioremediation Strategies

Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water). Bioaugmentation techniques involve the addition of microorganisms with the ability to degrade pollutants. It frequently involves the addition of microorganisms indigenous or exogenous to contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit. 1) non indigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and 2) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed. *In situ* bioremediation is generally the most desirable options due to lower cost and fewer disturbances since they provide the treatment in place avoiding exaction and transport of contaminants. *In situ* treatment is limited by the depth of the soil that can be effectively treated in some cases [90-96]. *Bioventing* is the most common *in situ* treatment and involves supplying nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface. In situ biodegradation involves supplying oxygen and nutrients by circulation aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment. *Biosparging* involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost installing small-diameter air injection points allows considerable flexibility in the design and construction of the system [95].

Ex situ bioremediation

This technique involves the excavation or removal of contaminated soil from ground. Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous Biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10-35 cm in soil. Since land farming has the potential ti reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative. Composting is a technique that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristics of composting. Biopiles are a hybrid of land farming and composting [96-100]. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favourable environment for indigenous aerobic microorganisms. Bioreactors- slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor mat be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid and gas) mixing condition to increase the bioremediation rate of soil bound and water soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganism) capable of degrading target contaminants. In general, the rate and extent of biodegradable are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predicable. Despite the advantages of reactors systems, there are some disadvantages. The contaminated soils require pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor [101-103].

Genetic engineering approaches

The use of genetic engineering to create organisms specifically designed for bioremediation has great potential. The bacterium *Deinococcus radiodurans* (the most radio resistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste. Most commonly, the process is misunderstood. The microbes are ever-present in any given context generally referred to as "normal microbial flora". During bioremediation (biodegradation) processes, fertilizers/nutrients supplementation is introduced to the environments, in efforts to maximize growth and production potential. Common misbelieve is that microbes are transported and dispersed into an unadulterated environment. *Micoremediation* is a form of bioremediation in which fungi are used to decontaminate the area. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by mycelium. The mycelium secretes extra cellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fibre. These are organic compounds composed of long chain of carbon and hydrogen, structurally similar to many organic pollutants [103,104]. Monitoring Bioremediation-The process of bioremediation can be monitored indirectly by measuring the Oxidation Reduction Potential or redox in soil and groundwater, together with pH, temperature, oxygen content, electron receptor/ donor concentrations and concentration of breakdown products e.g., CO_2 (Table 3).

Advantages of Bioremediation

Bioremediation is natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminants increase in numbers when the contaminant is present; when the contaminant is degraded, the bio degradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water and cell biomass. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example from land to water or air, the complete destruction of target pollutants is possible.

Processes	Reactions	Redox potentials (Eh in Mv)
Aerobic	$O_2+4e^-+4H^+ \rightarrow 2H_2O$	600~400
Anaerobic		
Denitrification	$2NO_3^-+10e^-+12H^+ \rightarrow N_2+6H_2O$	500~200
Manganese IV reduction	$MnO_2+2e^-+4H^+ \rightarrow Mn^{2+}$+2H2O	400 ~ 200
Iron III reduction	$Fe(OH)_3+3e^-+H^+ \rightarrow Fe^{2+}+3H_2O$	300~ 100
Sulphate reduction	$SO_4^{--}+8e^-+10H^+ \rightarrow H_2S+4H_2O$	0~150
Fermentation	$2CH_2O \rightarrow CO_2+CH_4$	150~220

Table 3: Reactions and Redox Potentials of some processes.

Technology	Examples	Benefits	Limitations	Factors to consider
Insitu	Bio remediation Biosparging Bioventing Bioagumentation	Most efficient, Non invasive, Relatively passive Natural attenuation, Process treat soil and water	Environmental Constraints, Extended treatment time difficulties	Biodegradative abilities of indigenous micro organism, Presence of metals &other inorganic, Environmental parameters, Biodegrability of pollutants, Chemical solubility, Geological factors, Distribution of pollutants
Exsitu	Land farming Compositing Biopiles	Cost efficient, Low cost, can be done on site	Space requirements, Extended treatment time, Need to control abiotic loss, Mass transfer problem, Bio availability limitation	As above
Bio reactors	Slurry reactors Aqueous reactors	Rapid degradation, Kinetic optimized, environmental Parameters, Enhances mass transfer effective, use of inoculants and surfactants	Soil requires excavation, Relatively high cost capital, Relatively high operating cost	Toxicity of amendments, Toxic concentration of contaminants

Table 4: Pros & Cons of Bioremediation.

- Bioremediation can often be carried out on site, often without causing a major disruption or normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
- Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste [104].

Disadvantages of Bioremediation

Bioremediation is limited to those compounds that are biodegradable, not all compounds are susceptible to rapid and complete degradation (Table 4).

- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound
- Biological processes are often highly specific. Important site factors required for successes include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants
- It is difficult to extrapolate from bench and pilot scale studies to full-scale field operations
- Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids and gases
- Bioremediation often takes longer time than other treatment options, such as excavation and removal of soil or incineration.
- Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of „clean", evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments.

Conclusion

The emerging of recent studies in molecular biology and ecology offers opportunities for more efficient biological processes to detoxify contaminants. Notable accomplishments of these studies include the clean-up of polluted water and land areas. Bioremediation is far less expensive than other technologies that are often used to clean up hazardous waste. Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, bio stimulation, and Bioagumentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behaviour of soil microorganisms that are indigenous to soil. Bio stimulation also utilizes indigenous microbial populations to remediate contaminated soils. Bio stimulation consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Bioagumentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms. There are a number of cost or efficiency advantages to bioremediation which can be employed in areas that are inaccessible without excavation. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable. However, as the range of contaminants on which it is effective is limited, the same time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. Because bioremediation seems to be a good alternative to conventional clean-up technologies research in this field is to be explored with advanced innovations.

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