

Bioremediation of Nitro-aromatics: An Overview

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Abstract— Since last two three decades due to industrialization, globalization there is tremendous change in human life that means to fulfil the need various industries are flourishing. We are facing the problem of environmental pollution and also facing hazards to biodiversity. So it becomes our duties to remediate the environment by using scientific tool like bioremediation. This is emerging as an effective innovative technology for treatment of a wide variety of contaminants. Bioremediation involves various approaches like phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Bioremediation is most effective technology for treatment of soil and water which are mostly contaminated by human activities. It is an economical process that means operation cost is less. In current review contamination of water and soil by nitro-aromatic compounds and the role of bacteria and fungi and their enzyme activity to enhance bioremediation process is studied by literature review. Nitro-aromatic compounds are used worldwide as explosives, pesticides and as a feedstock for the manufacture of many products, including dyes, pharmaceuticals, fungicides and plastics. On the contrary, nitro-aromatic compounds are released into the biosphere exclusively from the anthropogenic sources. Nitro-aromatic compounds do not only come from manmade sources; they also are formed by some natural processes, such as photochemical reactions in the atmosphere. Extensive production and indiscriminate application of nitro-aromatic has led to environmental pollution. Hence, nitro-aromatic compounds are recognized as Hazardous Rating-3.

Keywords— Bioremediation, enzyme, nitro-aromatic compounds, phytoremediation, rhizoremediation.

I. INTRODUCTION

One of the major environmental problems examined by the world today is the contamination of soil, water and air by toxic chemicals. Eighty billion pounds of hazardous organopollutants are produced annually and only 10% of these are disposed of safely. The estimated for their decontamination using traditional approaches such as incineration and landfilling is approximately one trillion [1]. Nitro-aromatic compounds are toxic to plant, animal and human health and poses significant health and

environmental risk due to its mutagenic and carcinogen activity. Nitro-aromatic compounds are generally considered to be highly resistant to microbial degradation. The purification of wastewater contaminated with these pollutants is very difficult since they are resistant to the conventional treatment techniques. Although several investigators have used physical and chemical methods such as volatilization, photodegradation, photo-catalysis and advanced oxidation to treat the wastewater containing nitro-aromatic compounds.

1.1 Bioremediation: a brief introduction

The term bioremediation was firstly introduced by scientists in early 1980s. Bioremediation is the use of the living organism to reduce or eliminate environmental pollution by various hazardous chemicals. Bioremediation involves transformation of complex or simple chemical compounds into nonhazardous forms by microbes [2]. The past two decades have seen a tremendous upsurge in the search for cost-effective and environmentally benign alternatives to the conventional methods for remediation of hazardous wastes. The use of microbes to clean up polluted environments is a rapidly changing and escalating area of environmental biotechnology. The explanation for their remarkable range of degradative abilities is that, by the time human beings came on the scene, microbes had already coexisted for billions of years with an immense variety of pollutants. The vast diversity of potential substrates for growth led to the evolution of enzyme capable of transforming many unrelated natural pollutants by many different catalytic mechanisms. The resulting giant 'library' of microbial enzymes serves as raw material for further revolution whenever a new chemical becomes available [3]. Being eco-friendly, this mode is sustainable too. The general approaches to bioremediation are basically (i) intrinsic bioremediation, (ii) biostimulation and (iii) bioaugmentation.

II. NITRO-PHENOLS: A BRIEF ACCOUNT

Nitro-phenols (NPs) are among the most important and versatile industrial organic compounds with applications as ingredients in pesticides, pharmaceuticals, pigments, dyes, and rubber chemicals [4]. Among the mono-nitrophenols, *p*-nitrophenol (PNP, also known as 4-

nitrophenol or 4-NP), is the most common and important in terms of quantities manufactured and extent of environmental contamination. PNP is (i) used for synthesis of medicines, dyes, explosives, leather colouring, wood preservatives, and rubber chemicals [5] and (ii) generated during formulation, distribution, and field application of pesticides or photodegradation of pesticides that contain the nitro-phenol moiety [6]. Consequently, PNP has often been detected in wastewater, rivers, soils, and ground water. Most nitro-phenols, including PNP and 3-nitrophenol, enter the environment through manufacturing and processing. Nitro-phenols are toxic to plants, microorganisms, animals and humans [7, 8]. The US EPA (1980) lists *p*-nitrophenol and 2-nitrophenol as priority pollutants, and restricts their concentrations in natural waters to 10 ngL⁻¹ [9]. The presence of substituted groups, i.e., nitro- and chloro-, on phenols increases the toxic effects on ecosystem and human health due to their persistence in the environment. Most of these compounds are resistant to microbial degradation, especially at high concentrations.

2.1 *p*-nitrophenol(PNP)

p-nitrophenol (PNP), a priority environmental pollutant, occurs in industrial effluents posing esthetic and health problems [10]. *p*-nitrophenol (PNP) was first registered in the United States in 1963 for use as a fungicide to control fungal mold on leather. In 1980, its application was registered for the protection of leather and military products at a concentration of 0.7% of dry leather weight. Its use was further extended for treatment of cork used in missile silo construction [11, 12]. It is primarily used as (i) solvent, (ii) commodity chemical for the synthesis of azo and sulphur dyes, (iii) explosives, (iv) number of intermediates, (v) pesticides (parathion, methyl parathion), (vi) insecticides (carbofuran, phosphalon, flurodifer), (vii) herbicides (nitrofen, bifernose), (viii) tubercutostatic 4-aminosalicylic acid, (ix) analgesic (4-acetoaminophenol/ paracetamol). PNP is manufactured on large scale (20 million Kg per annum) by many companies from Europe, USA and Japan. In 1996, environmental release of PNP as reported by USA manufacture was 45-450 MT per year. Its hazardous consequences are summarized in Table 1.

2.1.2 Toxicity

p-nitrophenol is a corrosive eye irritant (Toxicity Category I, indicating the greatest degree of acute toxicity) and a potential dermal irritant. *p*-nitrophenol is acutely toxic (Toxicity Category II) via the oral route and moderately toxic (Toxicity Category III) via the dermal route.

A subchronic oral toxicity study in rats showed an increased incidence of acute mortality, while a dermal study in mice resulted in dermal irritation and mortality. Chronic toxicity has not been conclusively evaluated. *p*-nitrophenol has been classified as Group D for carcinogenicity, indicating that there is inadequate information to determine its cancer potential. *p*-nitrophenol is not believed to cause reproductive or developmental toxicity, but additional studies are needed to confirm these tentative findings [12].

Since *p*-nitrophenol is classified as Toxicity Category I for eye irritation potential and since data on skin irritation potential are not available, the Agency is imposing risk reduction measures including use of personal protective equipment (chemical-resistant gloves and apron, and protective eyewear) as well as a long sleeved shirt, long pants, shoes and socks [12].

III. BIOLOGICAL SOLUTION TO THE POLLUTION

Nitro-aromatic compounds, now a days are recognized as high risk contaminants. Hence, their removal from environment is the first priority. Chemical approach may offer a temporary solution and may pose environment threat. On the contrary, microbial system is ubiquitous and versatile. It is well known that microbial entity plays central role in nutrient cycling on this planet [13]. Current routes for amelioration therefore, employ microorganisms which either immobilize or transform contaminants to innocuous end products. The incredible metabolic versatility of microbes permits them to (i) inhabit in hostile ecological niches and (ii) exploit compounds as a source of carbon, nitrogen and energy. Such microbial metabolic potential must be harnessed to develop process (es) for detoxification of recalcitrant(s). The strategy [14] offers many advantages like

Table.1: Hazardous consequences of nitro-aromatic compounds [15]

F-hazard/Exposure	Acute hazard/Symptoms	Prevention	First Aid/Fire Fighting
1. Fire	Combustible	No open flames, no contact with oxidant.	Powder, water spray, foam, carbon dioxide.
2. Explosion	Risk of Fire and explosion on contact with acid(s), oxidant.	Deposition of dust, close system, dust	In case of fire: keep drums etc. cool by

3. Exposure		explosion. Proof electrical equipment and lightening. Prevent dispersion of dust! Strict hygiene	spraying with water.
4. Inhalation	Blue lips/fingers, nails, blue skin, cough. Burning sensation, confusion, convulsion, dizziness, headache, nausea, sore throat, unconsciousness, weakness.	Local exhaust or breathing protection.	Fresh air, rest, refer for medical attention, artificial respiration may be needed.
5. Skin	May be absorbed, redness(further sea inhalation)	Protective gloves, protective clothing.	Remove contaminated clothes. Rinse and then wash skin with water and soap. Refer for medical attention.
6. Eyes	Redness, Pain.	Safety spectacles face shield or eye protection in combination with breathing protection.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
7. Ingestion	Abdominal pain , sore throat, vomiting (sea inhalation)	Do not eat, drink or smoke during work.	Rinse mouth, rest refer for medical attention.
8. Spoilage disposal	Packaging and labeling Sweep spilled substances into sealable container; if appropriate, moistened first to prevent dusting. Carefully collect remainder then remove to safe place. Do Not let this chemical enter the environment personal protection: P2 filter respirator for harmful particles.		

Operability in situ (ii) permanent elimination of contaminants through either biochemical transformation or cometabolic or mineralization, (iii) avoiding harsh physical and chemical treatment(s), (iv) cost effectiveness and (v) more public acceptability.

3.1 Bacteria

Microbial degradation of *p*-nitrophenol has been described for several genera including *Flavobacterium*, *Pseudomonas*, *Moraxella*, *Arthrobacter* and *Bacillus* [16]. *Bacillus sphaericus*, isolated from an agricultural soil by selective enrichment, transform *p*-nitrophenol [17]. A strain of *Pseudomonas putida* was found to degrade *p*-nitrophenol as a sole source of carbon, nitrogen and energy [18]. *Pseudomonas* sp and *Rhodococcus opacus* can utilize *p*-nitrophenol as a sole source of carbon and energy [19]. *Rhodococcus wratislaviensis* strain capable of utilizing *p*-nitrophenol as the sole source of carbon and energy and release the nitro group from the compound as

nitrite. The nitro group of PNP enhances the resistance of the aromatic ring to biodegradation, bacterial strains able to utilize PNP as a sole carbon and nitrogen sources include species of *Bacillus* [19], *Burkholderia* [20] and *Sphingomonas* sp. and *Sphingomonas chlorophenolica* strains can transform *p*-nitrophenol [21].

3.2 Fungi

Fungi have been used from fermentation of foods to production of pharmaceuticals. Fungi thrive well in inhospitable habitats with environmental extremes because of their enzyme system [22]. Fungi are involved in the biodegradation of undesirable materials or compounds and convert them into harmless, tolerable or useful products. Many organisms are involved in the biodegradation of organic waste, which has resulted in the production of novel substances of biotechnological importance.

Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints [23]. Fungi not only produce various metabolites like citric acid, homogeneous proteins, heterogeneous proteins, peroxidases but have shown their effectiveness for removal, reduction and detoxification of industrial effluents ingredients. Therefore, an attempt has been made to bring out the capabilities of fungi for bioremediation of industrial effluents. [24] have used filamentous soil fungi like *Aspergillus terreus*, *Cladosporium cladosporioides*, *Fusarium oxysporium*, *Gliocladium roseum*, *Penicillium* spp. and *Trichoderma koningii* isolated from industrially polluted sediments for the removal of Cadmium. The fungus *Penicillium frequentans* has been found to effectively remove phenanthrene in soil [25].

Fungi especially the white-rot fungi produce enzymes viz. laccase, Manganese peroxidase (MnP) and lignin peroxidase (LiP), which are involved in degradation of lignin in their natural lignocellulosic substrates. This ligninolytic system of white rot fungi is directly involved in the degradation of various xenobiotic compounds and dyes. The ability of the white rot fungi to degrade dye can be directly correlated with its ability to degrade lignin; the dye molecules are degraded along with lignin. Use of white rot fungi is the most unique technology of bioremediation as their ability to degrade structurally diverse xenobiotic organopollutants is more [26].

3.3 Fungi in biodegradation of pollutants

Biodegradation is an application of biological processes to the treatment of pollution. Most research within the field of bioremediation has focused on bacteria, with fungal bioremediation (mycoremediation) attracting interest just within the past two decades. White rot fungi is a physiological grouping of fungi that can degrade lignin (and lignin-like substances). Four main genera of fungi have shown potential for bioremediation *Phanerochaete*, *Trametes*, *Bjerkandera* and *Pleurotus* [27]. These fungi cannot use lignin as a source of energy, and instead require substrates such as cellulose or other carbon sources. Thus, carbon sources such as corncobs, straw, and sawdust can be easily used to enhance degradation rates by these organisms at polluted sites. Also, the branching, filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil.

3.4 Fungal mechanism of biodegradation

The main mechanism of biodegradation employed by this group of fungi, however, is the lignin degradation system of enzymes. These extracellular lignin-modifying enzymes (LMEs) have very low substrate specificity so they are

able to mineralize a wide range of highly recalcitrant organopollutants that are structurally similar to lignin [28]. The fact that these fungal enzymes work extracellularly allows them to access many of the non-polar, non-soluble toxic compounds that intracellular processes (such as cytochrome P450) cannot [29].

IV. CONCLUSION

This review focuses on various features in bioremediation processes and how the process acts as a biological tool for remediation of environment. It also encompasses the role of bacteria and fungi, their enzymatic activities. Advances in biotechnology, bioremediation has become a rapidly growing area. Selection of most appropriate strategy to treat hazardous chemicals and contaminated sites by microbial action is still needed.

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